“Environmental pollution is an incurable disease. It can only be prevented.”

Barry Commoner

Dissertation supervisors:

Prof. dr. ir. Nico Boon

Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

Prof. dr. ir. Siegfried E. Vlaeminck

Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

Department of Bioscience engineering , Faculty of Science, University of Antwerp, Belgium

Board of examiners:

Prof. dr. ir. Kristel Bernaerts

Bio- & Chemical Systems Technology, Reactor Engineering and Safety section, Department of Chemical Engineering (CIT), KU Leuven, Belgium

Prof. dr. Gavin Collins

Microbiology department, School of Natural Sciences, NUI Galway, Ireland

Dr. ir. Haydée De Clippeleir

DC Water and Sewer Authority, Washington, DC, United States

Prof. dr. ir. Ingmar Nopens (Secretary)

Department of Mathematical Modelling, Statistics and Bioinformatics, Faculty of Bioscience Engineering, Ghent University, Belgium

Prof. dr. ir. Paul Van Der Meeren (Chairman)

Department of Applied Analytical and Physical Chemistry, Faculty of Bioscience Engineering, Ghent University, Belgium

Prof. dr. ir. Stijn Van Hulle

Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University, Belgium

Dean Faculty of Bioscience Engineering:

Prof. Dr. ir. Marc Van Meirvenne

Rector Ghent University:

Prof. Dr. Anne De Paepe

Francis A. Meerburg

High-rate activated sludge systems to maximize recovery of energy from wastewater:

Microbial ecology and novel operational strategies

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Titel van het doctoraat in het Nederlands:

Hoogbelast actief slib voor het maximaliseren van energieherwinning uit afvalwater: Microbiële ecologie en nieuwe operationele strategieën

Cover illustration: Microscopic image of a floc of filamentous bacteria from a HiCAS reactor (see Chapter 3), magnified 630 times. The sludge is stained according to the Neisser procedure, which visualizes polyphosphate granules as purple dots. Picture by Tim Van Winckel; cover design inspired by Niels Lehouck.

Please refer to this work as:

Meerburg, F. A. (2016). High-rate activated sludge systems to maximize recovery of energy from wastewater: Microbial ecology and novel operational strategies. PhD thesis, Center for Microbial Ecology and Technology (CMET), Ghent University, Belgium.

ISBN: 978-90-5989-934-6

This work was funded by the Research Foundation Flanders (FWO-Vlaanderen). Additional support for reactor equipment was funded by the King Baudouin Foundation (Ernest du Bois prize).

Notation index

AB-system	Adsorptions-Belebungsverfahren
AD	Anaerobic digestion
AOA	Ammonium-oxidizing archaea
AOB	Ammonium-oxidizing bacteria
AerAOB	Aerobic ammonium-oxidizing bacteria
AnAOB	Anoxic ammonium-oxidizing bacteria
ANOVA	Analysis of variance
b	Endogenous decay rate
bCOD	Biodegradable chemical oxygen demand
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
BSA	Bovine serum albumin
C [chemical formulas]	Carbon
C [model expressions]	Colloidal substrate concentration
CA	Correspondence analysis
CAPEX	Capital expenditures
CAS	Conventional activated sludge
CCA	Canonical correspondence analysis
CEPT	Chemically enhanced primary treatment
CHP	Combined heat and power unit
COD	Chemical oxygen demand
CS	Contact stabilization
CSTR	Continuous stirred-tank reactor

$D_{4,3}$	Volume-weighted average diameter
DAF	Dissolved air flotation
DCB	Divalent cation bridging theory
DLVO	Derjaguin-Landau-Verwey-Overbeek theory
DO	Dissolved oxygen
DW	Dry weight
EBPR	Enhanced biological phosphorus removal
EPS	Extracellular polymeric substances
EPS.P	Proteinaceous extracellular polymeric substances
f	Fraction
F/M	Food-to-microorganism ratio
HiCAS	High-rate conventional activated sludge
HiCS	High-rate contact stabilization
HRAS	High-rate activated sludge
HRT	Hydraulic retention time
ISC	Initial settling classes
KjN	Kjeldahl nitrogen
k_La	Volumetric gas/liquid mass transfer coefficient
LOSS	Limit of stokesian settling
MBBR	Moving bed biofilm reactor
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MRM	Microbial resource management
N	Nitrogen
N/DN	Nitrification/denitrification

Nit/denit	Nitritation/denitritation
NOB	Nitrite-oxidizing bacteria
OPEX	Operational expenditures
OUR	Oxygen uptake rate
P	Phosphorus
PAO	Phosphorus-accumulating organisms
PE	Population equivalents
PE_{COD110}	PE based on a load of 110 g COD PE^{-1} d^{-1}
PHA	Poly-β-hydroxyalkanoates
PHB	Poly-β-hydroxybutyrate
Phy	Phylotype
PN/A	Partial nitritation/anammox
q	Sludge-specific substrate uptake rate
Q	Flow rate
qPCR	Quantitative real-time polymerase chain reaction
R.factor	Recirculation factor of final effluent back to influent
RDN	Regeneration-denitrification-nitrification process
RO	Reverse osmosis
rpm	Revolutions per minute
S	Soluble concentration
SBR	Sequencing batch reactor
SCP	Single-cell protein
SLR	Sludge-specific loading rate
SMP	Soluble microbial products
SOUR	Sludge-specific oxygen uptake rate
SRT	Solids retention time

SSE	Sum of squared errors
STP	Sewage treatment plant
SVI	Sludge volume index
t	Time
T	Temperature
t_c:t_s	Contact time : stabilization time ratio
TOD	Total oxygen demand
TOF	Threshold of flocculation
TS	Total solids
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
UCT	University of Cape Town process
V	Volume
VFAs	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant
X	Solids concentration
Y	Sludge yield

List of sub- and superscripts

ANO	Autotrophic nitrifying organisms
B, b	Biodegradable
Bf	Fast biodegradable organics
Bs	Slowly biodegradable organics
coll	Colloidal
crit	Critical
diss	Dissolved
E	Unbiodegradable endogenous products
e, eff	Effluent
el	Electrical
endo	Endogenous
EPS	Extracellular polymeric substances
eq	Equilibrium
exo	Exogenous
h	Heterotrophic
i, inf	Influent
lim	Limiting
max	Maximum
min	Minimum
NB	Biodegradable organic nitrogen
NH	Ammoniacal nitrogen (NH_3, NH_4^+)
NO	Oxidized nitrogen (NO_2^-, NO_3^-)
nom	Nominal
O	Oxygen

obs	Observed
part	Particular
r	Recycle
rem	Removed
S, s	Substrate
SBR	Sequencing batch reactor
syst	System
T	At current temperature
tot	Total
sp	Species
sto	Storage polymers
U	Unbiodegradable
w	Waste

Glossary

Following is a non-exhaustive list of terms. The definitions presented here are used throughout this manuscript. Different authors may maintain different definitions.

A-stage	HRAS process as the first stage of a wastewater treatment system with two or more stages. Traditionally operated in a HiCAS configuration, unless otherwise specified.
Accumulation	Intracellular uptake of substrate without chemical modification or minor modification to low-molecular weight intermediates.
Adsorption	Adhesion of particulate, colloidal and soluble organics onto the surface of microbial flocs.
B-stage	The second stage of a wastewater treatment system with two or more stages. Traditionally operated as a low-rate CAS process.
Bioflocculation	Biomass-mediated formation of larger flocs from smaller particules or colloids. May be used to denote flocculation of biological sludge in general, or to denote the removal of particulate and colloidal substrates by means of adsorption and enmeshment into the floc structure.
Biosorption	Collective term for the processes of non-destructive substrate uptake (i.e., accumulation, adsorption, and storage).
CAS	(Low-rate) conventional activated sludge. This configuration consists of a single contact phase between influent and return sludge, followed by solid/liquid separation. The contact phase may be subdivided into anaerobic, anoxic, and aerated phases to accommodate nitrogen and phosphorus removal, and may include internal MLSS recycling. A low-rate process is generally defined as having a sludge-specific loading rate below 0.6 g bCOD g^{-1} VSS d^{-1} and a solids retention time above 3 d.
Coagulation	Chemical destabilization and aggregate formation of particulate and colloidal matter by overcoming the electrostatic repulsion caused by the surface charge. Usually achieved by adding chemical coagulants to interact with the particle surface.

Deterministic change	Change in the structure or function of a microbial community under the influence of external (e.g., environmental) factors. The concept of deterministic change stems from the niche theory, which assumes that each microbial species possesses an optimal growth niche, and a change in environmental conditions will cause a deterministic (i.e., predictable) shift in species assembly as the growing conditions become more suitable for species with a slightly different niche. Antonym: neutral change.
F/M ratio	Food-to-microorganism ratio. While literature often expresses the F/M ratio in units of g BOD g^{-1} VSS d^{-1}, this is actually a rate, and is referred to as the sludge-specific loading rate (SLR) in this work. Here, the F/M ratio is meant as an actual ratio, in units of g BOD g^{-1} VSS, or similar units.
Feast-famine	Operational strategy where sludge is cyclically subjected to phases of high substrate availability (feast) and low substrate availability (famine). In principle, feast-famine regimes can be applied for any substrate. In this work, it is solely used for total COD as a substrate.
Flocculation	Physical adhesion of smaller aggregates to form larger flocs. Due to the relatively weak attraction forces, this process can easily be reversed (deflocculation).
HiCAS	High-rate conventional activated sludge. This configuration consists of a single contact phase between influent and return sludge, followed by solid/liquid separation. The contact phase may be subdivided into anaerobic, anoxic, and aerated phases.
HiCS	High-rate contact stabilization. This configuration consists of an aerated phase of stabilization of the return sludge, followed by a non-aerated or low-aerated phase of contact between influent and stabilized sludge, and solid/liquid separation.
HRAS	High-rate activated sludge. A high-rate process is generally defined as having a sludge-specific loading rate above 2 g bCOD g^{-1} VSS d^{-1} and a solids retention time below 2 d.
Neutral change	Change in the structure or function of a microbial community due to stochastic (i.e., chance-driven) processes. The concept of neutral change stems from the theory of island biogeography, in which an 'island community' (e.g., the microbial community in a bioreactor) is shaped by an equilibrium between extinction and colonization, as the

	random fluctuations in species abundance may cause some to go extinct and be replaced by other species that enter the system. Neutral change is nondirectional and cannot be precisely predicted. Antonym: deterministic change.
Storage	Intracellular uptake of substrate with chemical modification to form storage polymers.

TABLE OF CONTENTS

ABSTRACT (ENGLISH)

Water is a requirement to life and all human activity. Where water is used, wastewater is often created. Depending on the source, wastewater contains a range of pollutants, such as pathogens, organic matter, nitrogen, and phosphorus. Efficient treatment of wastewater is of the utmost importance to avoid public health problems, oxygen depletion in natural waters, and eutrophication. Conventional activated sludge (CAS) processes are commonly used to treat wastewaters, and although good removal efficiencies can be reached, CAS processes have a high electricity demand, high costs, and limited potential for recovery of resources such as energy and nutrient fertilizers.

To progress toward energy self-sufficient wastewater treatment systems, technologies need to be developed that radically improve their overall energy balance. Energy can be recovered from wastewater by anaerobic digestion (AD) of the sludge that is produced during the treatment process. During AD, biogas is formed, which can be combusted to produce electricity. To increase the recovery of energy from wastewater, a maximal amount of organic matter, which is measured as chemical oxygen demand (COD), needs to be redirected to sludge, instead of oxidized to CO_2. A number of strategies exist to lower the overall oxidation of COD and increase redirection to sludge. High-rate activated sludge (HRAS) systems produce more sludge with a better digestibility compared to conventional, low-rate systems. HRAS systems are operated at a short solids retention time (SRT, ≤ 2 d) and a high sludge-specific loading rate (SLR, 2 to 10 g bCOD g^{-1} VSS d^{-1}). Because HRAS systems typically do not remove sufficient amounts of COD and nutrients to allow direct discharge of the effluent, they are most valuable as the first stage in a two-stage treatment plants, where residual COD and other pollutants can be removed from their effluent during secondary treatment. Despite the fact that HRAS plants have been operated worldwide for decades, many plants struggle with operational problems related with a disturbance of the sludge microbial community. Little is known about the microbial ecology of HRAS systems, or how high-rate microbial communities differ from low-rate communities. There is a need for a more profound understanding of the processes that influence the high-rate community, so that strategies can be developed to better control the functional output of HRAS systems.

In the first part of this PhD (**Chapter 2**), the high-rate and low-rate communities of a two-stage municipal wastewater treatment plant were investigated, in relation to environmental and operational variables over a period of ten months. It was demonstrated that (1) high-rate and low-rate microbial communities are distinctly different in terms of richness, evenness and composition, (2) high-rate community dynamics are more variable and less shaped by deterministic factors compared to low-rate communities, (3) sub-communities of continuously abundant and transitional members are more shaped by deterministic factors than the continuously rare members, both in high-rate and low-rate communities, and (4) high-rate community members showed a co-occurrence pattern similar to that of low-rate community members, but were less likely to be correlated to environmental and operational variables. These results showed that important differences exist between high-rate and low-rate communities. Further research should investigate how these differences impact the functional output of HRAS systems. Ultimately, an improved knowledge of the

microbial ecology of high-rate systems should provide a basis for further optimization, and facilitate resource recovery from wastewater.

In the second part, operational strategies were explored to further improve COD recovery from wastewater. One option to achieve this, is by adopting a feast-famine regime, in which activated sludge is cyclically subjected to phases of high COD availability (feast) and low COD availability (famine). In a feast-famine regime, the activated sludge experiences a selection pressure for increased adsorption and storage, two mechanisms by which COD can be removed from wastewater and captured by the sludge without being immediately oxidized to CO_2. In this PhD thesis, it was proposed to further improve the adsorption and storage capacity of HRAS sludge by subjecting it to a feast-famine regime, in a so-called high-rate contact stabilization (HiCS) process. The HiCS process was hypothesized to combine the advantages of a high-rate process (i.e., a high sludge production rate and low percentage of COD oxidation) with those of a feast-famine regime (i.e., an increase of the adsorption and storage capacity of the sludge), to result in a better overall COD recovery performance compared to conventional high-rate processes. Furthermore, the HiCS system was hypothesized to have a lower volume requirement, which could result in lower overall investment costs compared to a conventional high-rate system.

In **Chapter 3**, the COD recovery performance of the HiCS system was compared to those of conventional systems in a laboratory-scale setup, using high-strength synthetic and low-strength real wastewater. Three HiCS reactors were operated at high SLR (>2 kg bCOD kg^{-1} TSS d^{-1}) and low SRT (<1.2 d). It was shown that the HiCS system could potentially recover more COD from wastewater organics than high-rate conventional activated sludge (HiCAS) and the low-rate contact-stabilization and CAS systems. The best HiCS reactor recovered 36% of the influent COD as methane, due to the combined effects of low production of CO_2, high sludge yield, and high methane yield during AD of the produced sludge. These results showed that, given further optimization, HiCS is a promising process for energy recovery from wastewater.

In **Chapter 4**, the HiCS process was optimized for COD recovery, and different biological pathways of COD removal were characterized. Eight HiCS laboratory-scale reactors were operated on high-strength synthetic wastewater, and operated at different combinations of SRT (0.24-2.8 d), hydraulic contact times (t_c, 8 and 15 min), and stabilization times (t_s, 15 and 40 min). The best performance was obtained at an optimal SRT between 0.5 and 1.3 d, a t_c of 15 min, and a t_s of 40 min, where the HiCS system oxidized only 10% of influent COD and recovered up to 55% of incoming organic matter into sludge. Storage played a minor role in the overall COD removal, which was likely dominated by aerobic biomass growth, bioflocculation onto extracellular polymeric substances and settling. It was calculated that the HiCS process recovered enough organics to potentially produce 28 kWh of electricity per population equivalent per year after AD of the sludge and electricity generation. This indicated that the HiCS process may substantially contribute to the development of energy-neutral wastewater treatment.

The aim of **Chapter 5** was to quantify differences in the overall energy expenditure and costs of a full-scale wastewater treatment plant when different primary and secondary treatment technologies were used. Reactor experiments were performed on medium-strength synthetic wastewater. The reactor configuration was switched from HiCAS to HiCS, and back to HiCAS, to quantify the COD recovery performance in these configurations, and assess whether the obtained performances are repeatable. The HiCAS and HiCS reactors had a similar COD recovery performance of 39% and 43%, respectively, but the HiCAS system oxidized up to 33% of COD, while the HiCS system oxidized only 7% and left a larger fraction of COD in the effluent. Batch experiments were performed to quantify the beneficial effect of coagulant addition on the COD recovery performance of a primary settling, HiCAS or HiCS unit. Subsequently, COD and nitrogen mass balance calculations were performed, and detailed energy, operational and investment costs were calculated for a two-stage wastewater treatment plant with different options for primary treatment (none, primary settling, HiCAS or HiCS), secondary treatment (nitrogen removal through nitrification/denitrification, nitritation/denitritation or partial nitritation/anammox), and side stream treatment (none or nitrogen removal from sludge digestate through partial nitritation/anammox). The scenarios with a HiCS system as primary treatment all reached a near-energy neutral overall wastewater treatment, with a net annual energy consumption of 0.98 to 1.08 kWh per population equivalent (PE), and had the lowest annual operational costs, at € 2.91 to 2.97 PE^{-1}, depending on the technology for secondary and side stream removal. Considering that the amortized investment costs to construct a HiCS system (€ 0.41 PE^{-1} y^{-1}) were offset by savings in operational costs compared to a conventional system without primary treatment, it may be economically favorable to retrofit a HiCS system into existing wastewater treatment plants. Chemical coagulation with $FeCl_3$ during primary treatment could help achieve energy positivity in all scenarios, but drastically increased operational costs. The economic advantage of the HiCS system compared to the HiCAS system was primarily due to the lower reactor volume requirements, and the fact that no external COD source was required for nitrogen removal. Additional work should assess the impact of further optimization of various factors along the wastewater treatment process, in order to further increase overall operational costs.

A final discussion addressed the roles of further research on microbial ecology, and technological optimization of COD recovery, toward the development of a more sustainable water-wastewater cycle. Preliminary investigations on the solid/liquid separation behavior of HiCS sludge, and progress in model-based research of the HiCS process, were presented. Overall, this work showed that high-rate activated sludge is a technology within reach to improve the overall energy balance of wastewater treatment. Given further improvement of the COD recovery performance, especially from medium- and low-strength wastewater, the HiCS process can be a preferred primary treatment technology for COD recovery in tomorrow's wastewater treatment plant.

SAMENVATTING (NEDERLANDS)

Water is een vereiste voor het leven en voor alle menselijke activiteit. En waar water wordt gebruikt, wordt vaak afvalwater geproduceerd. Afvalwater bevat, afhankelijk van de bron, verontreinigingen zoals pathogenen, organisch materiaal, stikstof en fosfor. Een efficiënte behandeling van afvalwater is daarom van uiterst belang om problemen te vermijden zoals gevaren voor de volksgezondheid, zuurstoftekorten in natuurlijke wateren, en eutrofiëring. Conventionele actief slib (CAS) processen worden algemeen gebruikt om afvalwater te zuiveren, en hoewel ze goede verwijderingsefficiënties kunnen bereiken, hebben CAS systemen een hoog elektriciteitsverbruik, hoge kosten, en een beperkte mogelijkheid om grondstoffen zoals energie en meststoffen uit het water te herwinnen.

Om te evolueren naar waterzuiveringssystemen die energetisch zelfvoorzienend zijn, is het nodig om technologieën te ontwikkelen die de totale energiebalans drastisch verbeteren. Energie kan worden herwonnen uit afvalwater via anaerobe vergisting (*anaerobic digestion*, AD) van het slib dat geproduceerd wordt tijdens het zuiveringsproces. Tijdens AD wordt biogas geproduceerd, dat vervolgens kan worden verbrand om elektriciteit op te wekken. Om de energieherwinning uit afvalwater te verhogen, moet een maximale hoeveelheid organisch materiaal, dat gemeten wordt als chemische zuurstofvraag (CZV), omgezet worden in slib, in plaats van geoxideerd te worden naar CO_2. Er bestaan een aantal strategieën die de oxidatie van CZV kunnen helpen verminderen en de omzetting in slib verhogen. Hoogbelast actief slib (*high-rate activated sludge*, HRAS) systemen produceren meer slib, met een betere vergistbaarheid, in vergelijking met conventionele, laagbelaste systemen. HRAS processen worden bedreven bij een korte slibretentietijd (SRT, ≤ 2 d) en een hoge slib-specifieke belasting (*sludge-specific loading rate*, SLR, 2 tot 10 g bCOD g^{-1} VSS d^{-1}). Omdat typische HRAS systemen niet voldoende CZV verwijderen om het effluent rechtstreeks te mogen lozen, zijn ze het meest nuttig als eerste trap in een tweetraps waterzuiveringsinstallatie, waar residuele CZV en andere verontreinigingen verder verwijderd kunnen worden in een secundaire behandeling. Hoewel HRAS systemen al tientallen jaren wereldwijd worden gebruikt, ondervinden vele installaties operationele problemen die te maken hebben met een verstoring van de microbiële gemeenschap van het slib. Er is weinig geweten over de microbiële ecologie van HRAS systemen, of over hoe de microbiële gemeenschap van een hoogbelast systeem verschilt van die van een laagbelast systeem. Er is nood aan een beter begrip van de processen die de hoogbelaste gemeenschap beïnvloeden, zodat controlestrategieën kunnen worden ontwikkeld om de functionele output van het systeem beter te controleren.

In het eerste deel van dit doctoraat (**Hoofdstuk 2**) werden de hoogbelaste en laagbelaste gemeenschappen van een tweetraps waterzuiveringssysteem voor stedelijk afvalwater onderzocht gedurende een periode van tien maanden, in relatie met omgevings- en operationele parameters. Er werd aangetoond dat (1) hoogbelaste en laagbelaste microbiële gemeenschappen uitgesproken verschillen vertonen in soortenrijkdom, gelijkmatigheid en samenstelling, (2) hoogbelaste gemeenschappen meer dynamisch zijn en minder worden beïnvloed door deterministische factoren, (3) de deelgemeenschappen van permanent abundante en transitionele soorten meer beïnvloed worden door deterministische factoren dan de deelgemeenschap van permanent zeldzame soorten, zowel in hoogbelaste als laagbelaste systemen, en (4) het patroon van gelijktijdigheid van voorkomen

van soorten in de hoogbelaste gemeenschap gelijkaardig is aan dat in de laagbelaste gemeenschap, maar dat soorten in de hoogbelaste gemeenschap een lagere waarschijnlijkheid hebben om gecorreleerd te zijn met omgevings- en operationele parameters. Deze resultaten tonen belangrijke verschillen aan tussen hoogbelaste en laagbelaste gemeenschappen. Verder onderzoek moet uitklaren hoe deze verschillen de functionele output van HRAS system beïnvloeden. De verbeterde microbieel-ecologische kennis van hoogbelaste systemen dient uiteindelijk een basis te vormen voor verdere optimalisatie, en om grondstoffenherwinning uit afvalwater te faciliteren.

In het tweede deel werden operationele strategieën onderzocht om de CZV-herwinning uit afvalwater verder te verbeteren. Eén van de mogelijkheden is het gebruik van een *feast-famine* regime, waarbij het actief slib cyclisch wordt blootgesteld aan perioden van hoge CZV beschikbaarheid (*feast*) en lage CZV beschikbaarheid (*famine*). In een *feast-famine* regime wordt het slib onderworpen aan een selectiedruk voor een betere adsorptie en opslag, twee mechanismen waarmee CZV kan worden verwijderd uit afvalwater en afgevangen in het slib zonder dat het meteen wordt geoxideerd tot CO_2. In deze doctoraatsthesis werd voorgesteld om de adsorptie- en opslagcapaciteit van HRAS slib verder te verbeteren door het aan een *feast-famine* regime te onderwerpen, in een zogenaamd hoogbelast contact-stabilisatie (*high-rate contact stabilization*, HiCS) proces. Er werd voorondersteld dat het HiCS proces de voordelen van een hoogbelast systeem (i.e., een hoge slibproductie en laag percentage CZV oxidatie) kan koppelen aan de voordelen van een *feast-famine* regime (i.e., een verbeterde adsorptie- en opslagcapaciteit), om te resulteren in een verbeterde totale herwinning van CZV in vergelijking met conventionele hoogbelaste systemen. Verder werd voorondersteld dat een HiCS reactor lagere volumevereisten heeft dan conventionele hoogbelaste reactoren, wat zou kunnen leiden tot een lagere investeringskost.

In **Hoofdstuk 3** werd de CZV-herwinning in het HiCS systeem vergeleken met die van conventionele systemen in een opstelling op laboschaal met synthetisch afvalwater met hoge sterkte, en echt afvalwater met lage sterkte. Drie HiCS reactoren werden gelopen aan een hoge SLR (>2 kg bCOD kg^{-1} TSS d^{-1}) en lage SRT (<1.2 d). Er werd aangetoond dat het HiCS systeem potentieel meer CZV kan herwinnen dan een hoogbelast conventioneel systeem (HiCAS) en de laagbelaste contact-stabilisatie en CAS systemen. De beste HiCS reactor kon 36% van de inkomende CZV herwinnen in de vorm van methaan, door de gecombineerde effecten van een laag oxidatiepercentage naar CO_2, een hoge slibproductie, en een hoge methaanproductie tijdens anaerobe vergisting. Deze resultaten tonen aan dat, na verdere optimalisatie, HiCS een beloftevol proces is om energy terug te winnen uit afvalwater.

In **Hoofdstuk 4** werd het HiCS systeem geoptimaliseerd voor CZV herwinning, en verschillende biologische mechanismen van CZV verwijdering werden gekarakteriseerd. Acht HiCS reactoren werden gelopen op laboschaal met synthetisch afvalwater met hoge sterkte, bij verschillende combinaties van SRT (0.24-2.8 d), hydraulische contacttijd (t_c, 8 en 15 min), en stabilisatietijd (t_s, 15 en 40 min). De beste prestaties werden geleverd bij een optimale SRT tussen 0.5 en 1.3 d, een t_c van 15 min, en een t_s van 40 min, waarbij slechts 10% van de inkomende CZV werd geoxideerd en 55%

herwonnen in de vorm van slib. Opslag droeg slechts in beperkte mate bij aan de CZV verwijdering, die voornamelijk bepaald werd door aerobe groei van biomassa, bioflocculatie van particulair materiaal, en bezinking. Er werd berekend dat de CZV herwinning van het HiCS systeem voldoende is om 28 kWh elektriciteit op te wekken per persoon per jaar, door middel van anaerobe vergisting. Dit toonde aan dat het HiCS systeem een substantiële bijdrage kan leveren aan de ontwikkeling van energetisch zelfvoorzienende afvalwaterzuivering.

Het doel van **Hoofdstuk 5** was om het totale energieverbruik en de totale kosten van een volle-schaal waterzuiveringsinstallatie te kwantificeren, voor verschillende primaire en secundaire behandelingstechnologieën. Er werden reactorexperimenten uitgevoerd op synthetisch afvalwater met medium sterkte. De configuratie van de reactoren werd gewisseld van HiCAS naar HiCS, en terug naar HiCAS, om de CZV-herwinningsefficiëntie te bepalen voor deze configuraties, en in te schatten of de bekomen prestaties herhaalbaar zijn. De HiCAS en HiCS reactoren behaalden een gelijkaardig herwinningspercentage van resp. 39% en 43% van de inkomende CZV, maar in het HiCAS systeem werd tot 33% CZV geoxideerd naar CO_2, terwijl het HiCS systeem slechts 7% oxideerde en de resterende CZV in het effluent terechtkwam. Batchexperimenten werden uitgevoerd om het verhogend effect te bepalen van coagulantdosering op de CZV-herwinningsefficiëntie van een primair bezinkingssysteem, een HiCAS en een HiCS systeem. Vervolgens werden massabalansen berekend voor CZV en stikstof, en werden gedetailleerde berekeningen van het energieverbruik, operationele kosten en investeringskosten uitgevoerd voor een tweetraps waterzuiveringsinstallatie met verschillende opties voor primaire behandeling (geen, primaire bezinking, HiCAS of HiCS), secundaire behandeling (stikstofverwijdering via nitrificatie/denitrificatie, nitritatie/denitritatie, of partiële nitritatie/anammox), en zijstroombehandeling (geen of stikstofverwijdering uit slibdigestaat via partiële nitritatie/anammox). Wanneer de primaire behandeling uit een HiCS systeem bestond, was de totale waterzuivering nagenoeg energieneutraal, met een jaarlijks verbruik tussen 0.98 en 1.08 kWh per populatie-equivalent (PE), en werden de laagste jaarlijkse operationele kosten bekomen (€ 2.91 tot 1.97 PE^{-1}), afhankelijk van de gebruikte technologie voor secundaire en zijstroombehandeling. De geamortiseerde investeringskosten voor de constructie van een HiCS systeem (€ 0.41 PE^{-1} y^{-1}) werden gecompenseerd door de besparingen in operationele kosten in vergelijking met een conventioneel systeem zonder primaire behandeling, waaruit bleek dat het economisch rendabel kan zijn om bestaande waterzuiveringsinstallaties om te bouwen tot tweetraps systemen met een HiCS proces. Dosering van $FeCl_3$ als coagulant resulteerde in een energiepositief waterzuiveringsproces voor alle scenario's, maar de operationele kosten werden drastisch verhoogd. Het economisch voordeel van het HiCS systeem in vergelijking met het HiCAS systeem lag voornamelijk aan de lagere volumevereisten, en aan het feit dat er geen externe CZV bron toegediend moest worden voor stikstofverwijdering. Bijkomend onderzoek moet zich richten op de impact van verdere optimalisatie van verschillende factoren langsheen het gehele waterzuiveringsproces, zodat de totale operationele kosten verder verlaagd kunnen worden.

In een finale discussie werd de rol besproken van verder onderzoek van de microbiële ecologie, en van verdere technologische optimalisatie van de CZV-herwinning, op de ontwikkeling van een meer

duurzame water-afvalwatercyclus. Preliminaire resultaten werden voorgesteld van onderzoek naar het slib-water afscheidingsgedrag van HiCS slib, en van de vooruitgang in het ontwikkelen van een modelmatige beschrijving van het HiCS proces. Algemeen toonde dit werk aan dat hoogbelast actief slib een bereikbare technologie is om de totale energiebalans van de waterzuivering te verbeteren. Na verdere verhoging van de CZV-herwinningsprestaties, in het bijzonder voor afvalwater van medium en lage sterkte, kan het HiCS proces de voorkeurstechnologie worden voor CZV herwinning in de waterzuivering van morgen.

CHAPTER 1:

INTRODUCTION

1 The anthropogenic water cycle

Water is the key to all things living. Organisms consist for a considerable part of water. A crucial prerequisite – direct or indirect – for nearly all human activity, water needs to be available wherever humans go. As societies grew during the course of history, they outgrew the capacity of local natural ecosystems to provide fresh drinking water and process watery wastes. Mankind needed to make more freshwater available and get rid of wastewaters faster, by developing technologies to alter and complement the natural water cycle. From the aqueducts in ancient Rome to the latest technological improvements in membrane technology, the anthropogenic water cycle has constantly evolved to become larger, more efficient and ever more reliable.

On a global average, today's anthropogenic water cycle is responsible for the withdrawal of 3900 km^3 of freshwater from the environment every year, of which 12% is for municipal use, 19% for industrial use and 69% for agricultural use (AQUASTAT, 2014). Zooming in on Western Europe, an annual water withdrawal of 99.7 km^3 y^{-1} accounts for 15.9% of the volume that is replenished every year by local precipitation. Of course, these are average numbers. More than half of European cities are withdrawing groundwater at unsustainably high rates (UN, 2006). A water-stressed region such as the Arabian peninsula withdraws water at a rate of almost five times the precipitation rate (AQUASTAT, 2014). By 2050, climate change and rapid socio-economic development are projected to increase water stress on river basins by about 50% (Alcamo et al., 2007). A global 1.1 billion people already lacked sufficient access to drinking water in 2006, and 2.6 billion people lacked access to basic sanitation (UN, 2006) which, in turn, can greatly reduce the availability of safe-to-drink freshwater. These examples illustrate the urgent need to transition toward a more sustainable anthropogenic water cycle.

Modern water systems may either be organized at a distributed or a centralized level, and each approach brings its own advantages and disadvantages to the table of transitioning towards a sustainable system. Distributed (i.e., decentralized) systems can be designed to treat, use and reuse water on a local scale and thus avoid the construction of expensive underground water and wastewater networks (van Lier & Lettinga, 1999). Furthermore, they can be made compatible with source separation for a more efficient recovery of resources. Their relative independency of centralized supply of drinking water, collection of wastewater, and even electricity supply, makes them less sensitive to crisis and supply chain instability. Distributed discharge of treated wastewater can avoid environmental problems related to single-point release of large volumes of water with residual contaminants, which may be an issue in centralized systems when the local ecosystem may not be capable of handling the flux of residual organic matter and nutrients (Libralato et al., 2012). Centralized water treatment, on the other hand, is still more economically interesting in case an underground sewer system already exists (Maurer et al., 2005). In densely populated areas, the economy of scale makes it more feasible to employ state-of-the-art technologies to remove contaminants and recover resources on a central level. However, the dependency on underground pipe networks comes with disadvantages such as losses of drinking water through pipe leaks, sewage

dilution by rainwater, runoff and groundwater infiltrating through leaks, septic contamination of groundwater through those same leaks, and intermixing of sewage with industrial wastewaters that contain difficult-to-remove contaminants (Libralato et al., 2012). During storms, centralized water infrastructure may cause additional environmental pollution and public health issues, both in places where a separate storm sewer system exists that carries untreated urban runoff and debris to a local water body, as well as in places where a combined sewer system exists that collects sewage and rain water, overflows during storms and bypasses the wastewater treatment plant (Metcalf & Eddy, 2003). When redesigning the water cycle of the future, a choice has to be made between distributed or central systems, depending on factors such as locality, population density, climatic conditions and the existing infrastructure, and this choice will strongly impact the efficacy and economic prospects of the water treatment technologies to be used (Sedlak, 2014).

2 Wastewater mining

Wastewater needs to be treated primarily because it contains pathogens, organic matter and nutrients. If wastewater would be directly discharged into the natural environment, these constituents would cause widespread public health problems, oxygen depletion in natural waters, and eutrophication. Conventional activated sludge (CAS) processes are a group of widely used wastewater treatment processes with acceptable removal efficiencies for pathogens, organic matter and nutrients. However, CAS processes have a high electricity demand and limited potential for recovery of resources such as energy and nutrient fertilizers. Much effort is directed to improve resource recovery from wastewater.

2.1 Recovery of wastewater resources

With global reserves of natural resources at risk of becoming economically scarce or even failing to meet demands, society is turning its eyes towards resource recovery from waste streams. With proper technology and control systems, domestic wastewater can be a source of chemical energy, nutrients, and drinking water. Reuse of wastewater resources is not a new idea; it has been practiced ever since humanity discovered that crops grow better with animal or human waste as fertilizer. Sewage farming was still extensively practiced deep into the first half of the 20th century, when commercialization of the Haber-Bosch process allowed farmers to switch to synthetic fertilizer (Orhon, 2015).

Recovery of energy in the Western world via anaerobic digestion (AD) found its roots at the start of the 20th century, but only several decades later, in the 1950s, was the process sufficiently understood to allow large-scale application of the AD process (Khanal, 2008). With the spreading of AD as an energy recovery technology came the realization that excess biological sludge should no longer be regarded as ‘waste’ but as a source of recovered energy. Soon after the development of the Adsorptions-Belebungsverfahren (AB-system) in the energy crisis of the 1970s (see section 5.3), it was recognized that AB-systems could recover more biological sludge for energy recovery while

achieving the same effluent quality compared to CAS systems (Böhnke, 1984). Today, systems inspired on the AB-process are still regarded as one of the most viable options to achieve efficient energy recovery from wastewater, because in all but a few cases where direct AD of raw wastewater is feasible (Smith et al., 2013; Verstraete et al., 2009), energy recovery always requires an up-concentration of wastewater organics before efficient conversion to biogas can take place. High-rate activated sludge (HRAS) is a preferred technology to achieve this, because the high loading rates and short solids retention times (SRTs) of HRAS systems result in a high fraction of incoming organic matter recovered as sludge instead of oxidized (see section 5.2). And with an A-stage comes a B-stage, because nutrient removal by an HRAS system alone is unsatisfactory for today's standards.

Regarding nutrients, recovery may be feasible if the nutrient-containing streams are relatively concentrated, and may include technologies such as air stripping and mineral precipitation from treatment plant side streams, as discussed in sections 3.2 and 3.3.

Reuse of wastewater for the production of drinking water is a more recent idea, if not considering the age-old but unavoidable practice of indirect reuse, e.g., when (treated) wastewater is discharged into a natural water body and taken up a few kilometers downstream for the production of drinking water. Planned introduction of treated wastewater into the drinking water supply system only became viable on a large scale after the invention and optimization of the reverse osmosis (RO) process in the 1950s and 1960s. RO can produce water of an unprecedented quality from wastewater treatment plant (WWTP) effluents at an acceptable operational cost. However, drinking water production from RO-treated WWTP effluent struggles with public acceptance and is only practiced in a limited number of places, such as Orange County, Los Angeles's West Basin and East Valley, and San Diego (California), El Paso (Texas), Arizona, Singapore, and Koksijde (Belgium) (Sedlak, 2014; Van Houtte & Verbauwhede, 2012), although in many of these systems, the reuse is indirect because the water passes a natural barrier before being used for drinking water production (e.g., groundwater recharge, dune filtration) or, in the case of Singapore, the water is used for non-potable purposes only.

2.2 Towards a sustainable water cycle

In recent years, efforts have been made to shift the paradigm of conventional wastewater treatment to a new approach where the above technologies are combined into a sustainable, integrated anthropogenic water cycle. These systems envision wastewater as a 'mine' from which drinking water, energy and nutrients can be recovered and short-cycled back into the economy – in other words, 'wastewater mining'. The possibilities for practical implementation of this philosophy are diverse and depend on the type of wastewater, the scale of the installation, and the fact whether different waste sources are collected separately or combined, such as blackwater, greywater, rain and runoff water, and even solid kitchen waste. For the case of centralized mixed municipal wastewater, a number of conceptual treatment schemes has been proposed to achieve maximal resource recovery from wastewater (Gao et al., 2014; Palmer & Nair, 2011; Scherson & Criddle, 2014; Verstraete et al., 2009; Verstraete & Vlaeminck, 2011; Wilsenach & van Loosdrecht, 2006), although

these studies differ in the extent to which they propose to recover different resources. The AB-system, while modernized as a combination of any high-rate technology to up-concentrate and recover organic carbon, and any low-rate technology for removal of residual nutrients, forms the backbone in many of these proposed schemes.

Of all resources, energy is perhaps the resource closest to becoming recoverable from wastewater on a large scale, because advanced wastewater treatment facilities are often already equipped with AD installations, and perhaps also because recovery of energy from wastewater does not involve public health regulations or require changes in supply chains, as may be an issue for drinking water and nutrients. Producing energy from wastewater, or at least reducing net energy consumption, is a central theme in many efforts to optimize wastewater treatment. Today, energy self-sufficient municipal wastewater treatment plants are extremely rare (Nowak et al., 2011; Wett et al., 2007), but achieving energy self-sufficiency should be possible for the majority of WWTPs, given some further optimization of existing technologies (De Clippeleir et al., 2015; Jenicek et al., 2013; Wett et al., 2007).

Apart from combining technologies for water, nutrients and energy reuse, other issues need to be addressed before a sustainable integrated anthropogenic water cycle can be achieved. These include, but are not limited to:

- Infrastructural choices, such as the choice between centralized or decentralized systems (see section 1) and the possibility of source separation
- Economical choices, such as the decision between the high expense of building new infrastructure versus using the existing but outdated sewer and distribution networks that may be more expensive in the long-term
- Environmental finance choices, such as the development of a revenue model that incorporates the avoidance of the high externalized costs of environmental damage associated with CAS systems
- Public acceptance issues, such as public involvement in the decision process (Guest et al., 2009) and the choice between voluntary compliance or a mandatory approach
- Public health issues, such as the need to develop methods to remove trace contaminants, heavy metals and disinfection by-products
- Environmental issues, such as the fate of final waste streams that cannot be recycled, or the importance to recognize that resource recovery technologies may have a high environmental footprint through emission of greenhouse gases (Mo & Zhang, 2012; Schaubroeck et al., 2015; Sweetapple et al., 2015)

3 Back to basics: activated sludge

The core technology of wastewater treatment is activated sludge. In the past 100 years since its conception (Ardern & Lockett, 1914; Clark & Adams, 1914; Clark & Gage, 1913), the activated sludge process has been modified and perfected, but its main principles remained the same. Activated sludge is a rich suspension of bacteria and other microorganisms that can be used to remove constituents from wastewater, such as organic matter (expressed as biological oxygen demand, BOD; and chemical oxygen demand, COD), suspended particles (expressed as volatile suspended solids, VSS; and total suspended solids, TSS), nitrogen (N), phosphorus (P), and other constituents such as pathogenic bacteria, viruses, metals and other trace contaminants. Successful removal of these constituents depends on the specific operating conditions of the activated sludge process. In its simplest form (**Figure 1.1**), activated sludge treatment consists of an aeration basin in which untreated 'raw' wastewater is fed to the sludge in a continuous stream – the influent. The aeration provides oxygen for the bacteria, which use the organic matter and nutrients of the wastewater as substrate for their growth. At the other side of the basin, the 'mixed liquor' – the mixture of treated wastewater and suspended sludge – flows to a settling basin, where sludge and particles settle to the bottom and are sent back to the activated sludge basin, while the clarified top layer leaves the system as the effluent and can be discharged. To keep the bacteria in the activated sludge from growing too abundant, a fraction of the settled sludge needs to be removed and processed as waste solids.

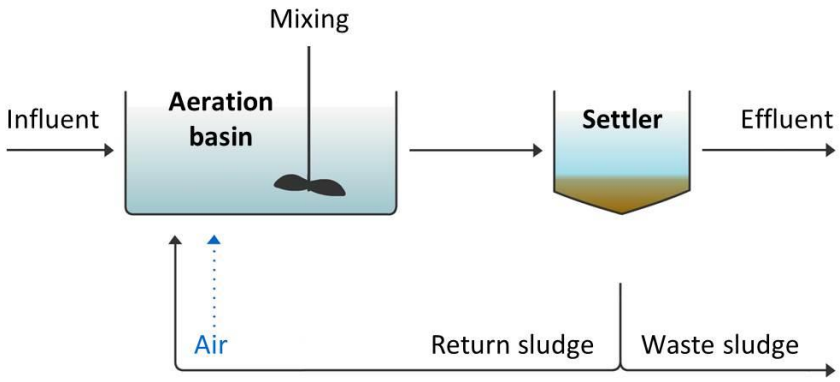


Figure 1.1: Schematic representation of a basic activated sludge process, after Meerburg et al. (2015).

Rarely, however, wastewater treatment is as simple as this. The process explained above may achieve an efficient removal of organic matter, but a large part of the nitrogen, phosphorus and other constituents will remain in the effluent at levels far above the stringent discharge standards set forth by governmental regulations. Most wastewater treatment plants (WWTPs) operate according to 'conventional activated sludge' (CAS) processes, in which a number of sub-processes are operated to target the removal of these constituents. The term is not strictly defined, but CAS processes

consist of a biological treatment basin, which can be sectioned into aerated and non-aerated parts to remove COD as well as nitrogen and possibly phosphorus, after which settlers separate the sludge from the effluent. Before biological treatment, CAS plants typically operate screens and sand traps to remove large debris and grit from the wastewater. In larger plants, the wastewater often goes through a primary settling phase, in which heavier particles settle down and scum, grease and oils can be removed from the floating top layer, after which biological treatment constitutes the secondary treatment phase. If phosphorus needs to be removed by chemical precipitation, chemicals are added before the primary or secondary settling step. In the following sections, different biological technologies for carbon, nitrogen and phosphorus removal are described.

3.1 Carbon removal

3.1.1 Carbon conversion reactions

The main groups of microorganisms in activated sludge are auto- and heterotrophs. While autotrophs derive their energy from inorganic reactions and use CO_2 as carbon source, heterotrophic bacteria use organic matter as energy and carbon source. It is thus the heterotrophs that are responsible of removing organic matter from wastewater. A general organic molecule with the formula $C_xH_yO_z$ will be partially oxidized to CO_2, protons and electrons according to following half-reaction (Van Haandel & van der Lubbe, 2007):

$$C_xH_yO_x + (2x - z)H_2O \rightarrow x\,CO_2 + (4x + y - 2z)H^+ + (4x + y - 2z)e^- \quad \text{Equation 1.1}$$

These electrons are used by the bacteria to produce energy, which requires an electron acceptor. In aerobic conditions, oxygen is the preferred electron acceptor:

$$e^- + H^+ + \frac{1}{4}O_2 \rightarrow \frac{1}{2}H_2O \quad (aerobic) \quad \text{Equation 1.2}$$

In anoxic conditions, i.e., in the absence of oxygen but the presence of other inorganic electron acceptors, the most important electron acceptors are nitrate (denitrification) and nitrite (denitritation):

$$NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2}N_2 + 3H_2O \quad (anoxic) \quad \text{Equation 1.3}$$

$$NO_2^- + 4H^+ + 3e^- \rightarrow \frac{1}{2}N_2 + 2H_2O \quad (anoxic) \quad \text{Equation 1.4}$$

Another part of the organic substrate will be used to create new biomass:

$$C_xH_yO_z + \frac{x}{5}NH_4^+ \rightarrow \frac{x}{5}C_5H_7O_2N + \left(z - \frac{2x}{5}\right)H_2O + \left(\frac{x}{5} + y - 2z\right)H^+ + (y - 2z)e^- \quad \text{Equation 1.5}$$

Where bacterial biomass is approximated by the empirical formula $C_5H_7O_2N$ (Hoover & Porges, 1952).

Wastewater contains a complex mixture of organic chemicals, including fats and fatty acids, proteins and amino acids, polysaccharides and carbohydrates, bacterial biomass, food residues, as well as a range of refractory compounds such as humic substances and synthetic chemicals (Metcalf & Eddy, 2003). Because of the impossibility of measuring and reporting the concentration of each of these compounds, organic carbon compounds are often expressed in terms of the amount of oxygen they can reduce, according to the electron flow of Equations 1.1 and 1.2. As such, the chemical oxygen demand (COD) is the amount of oxygen that can be reduced by the organic matter in the wastewater, and is usually expressed as mg oxygen per liter. However, because COD concentrations are measured chemically, they do not reflect how well the organic matter can be degraded by bacteria. To account for the fraction of difficult-to-degrade COD in wastewater treatment, wastewater is often characterized in terms of biodegradable COD (bCOD), which reflects the maximum fraction of COD that can be degraded biologically:

$$bCOD = COD \times f_b \qquad \text{Equation 1.6}$$

Where f_b is the biodegradable fraction, and differs according to the type of wastewater. The biodegradable fraction can be determined by adding a seed stock of microorganisms to a sample of wastewater and measuring the cumulative reduction of oxygen over time (Greenberg et al., 1992). This is the biochemical oxygen demand (BOD) and follows the relationship:

$$BOD_t^T = BOD^\infty \times \left(1 - e^{-kt}\right) \qquad \text{Equation 1.7}$$

Where BOD_t^T is the cumulative biochemical oxygen demand after time t at temperature T, BOD^∞ is the ultimate BOD and k is the biodegradation rate constant, which typically has a value of 0.23 d^{-1} (Metcalf & Eddy, 2003). BOD concentrations are typically measured after 5 days at a temperature of 20°C (BOD_5^{20}). During a BOD measurement, the bacterial biomass from the seed stock will grow and subsequently degrade, upon which some non-degradable residue of the biomass will remain. This so-called endogenous residue is the reason why the ultimate BOD slightly differs from the bCOD. For practical purposes, bCOD concentrations are often approximated by the empirical relationship $BOD_5 = 0.65 \times bCOD$. More exact is the following relationship:

$$BOD^\infty = bCOD \times \left(1 - Y_{h,max} \times f_d\right) \qquad \text{Equation 1.8}$$

Where f_d is the endogenous residue, for which a typical value is 0.10-0.15 (Metcalf & Eddy, 2003), and $Y_{h,max}$ is the maximum heterotrophic growth yield.

The maximum yield (Y_{max} or simply Y) is a central concept in biological wastewater treatment. It describes the division of substrate along the pathways of biomass growth (e.g., Equation 1.5) and energy metabolism (e.g., Equations 1.1 and 1.2), whereby Y is the fraction of substrate used for initial biomass production, and (1 – Y) is the fraction of substrate that is directly catabolized by the process of exogenous respiration. Biomass can be expressed in terms of COD, volatile suspended solids (VSS) or total suspended solids (TSS) and, accordingly, the yield is expressed as g $COD_{produced}$ g^{-1} $COD_{removed}$, g $VSS_{produced}$ g^{-1} $COD_{removed}$ or g $TSS_{produced}$ g^{-1} $COD_{removed}$. The heterotrophic yield depends on the type

of substrate. The most commonly accepted value for wastewater is $Y_{h,max}$ = 0.67 g COD g^{-1} COD or 0.44 g VSS g^{-1} COD in aerobic conditions, although reported values range between 0.58 and 0.67 g COD g^{-1} COD (Henze et al., 2000; Hvitved-Jacobsen et al., 1998; Ni et al., 2008). In anoxic conditions, the heterotrophic growth yield is slightly lower, with a typical value of 0.54 g COD g^{-1} COD (Henze et al., 2000). For other heterotrophic processes, such as substrate storage, yields can be higher than the heterotrophic growth yield (Dircks et al., 1999) and even approach the value of 1 if little or no energy is used for the process. In anaerobic conditions (i.e., in the absence of inorganic electron acceptors) heterotrophic processes tend to occur at much lower yields of around 0.08 g VSS g^{-1} COD (Metcalf & Eddy, 2003).

3.1.2 The kinetics of heterotrophic growth

The yield factor can be used to estimate biomass production according to the kinetic equation

$$\mu = Y_{h,max} \times q - b \qquad \text{Equation 1.9}$$

Where μ is the specific biomass growth rate (g $COD_{produced}$ g^{-1} $COD_{biomass}$ d^{-1}), q is the specific substrate uptake rate (g $COD_{removed}$ g^{-1} $COD_{biomass}$ d^{-1}) and b is the specific endogenous decay rate (g $COD_{decayed}$ g^{-1} $COD_{biomass}$ d^{-1}). Substrate uptake sometimes occurs under conditions that are sub-optimal for bacterial growth, such as low substrate concentrations and low oxygen concentrations. To account for changes in growth rate depending on how close the system is to optimal conditions, the substrate uptake rate can be expressed as a fraction of the maximal substrate uptake rate, using Monod expressions (Monod, 1949):

$$q = \hat{q} \times \frac{S_S}{K_{S,H}+S_S} \times \frac{S_O}{K_{O,H}+S_O} \qquad \text{Equation 1.10}$$

Where q̂ is the maximum specific substrate uptake rate, S_S and S_O are the substrate and oxygen concentrations, respectively, and $K_{S,H}$ and $K_{O,H}$ are the half-saturation constants for substrate and oxygen for heterotrophs, i.e., the respective concentrations of substrate or oxygen at which the substrate uptake rate will be half its maximum. By combining Equation 1.9 with Equation 1.10, the specific biomass production rate can be expressed as:

$$\mu = Y_{max} \times \hat{q} \times \frac{S_S}{K_{S,H}+S_S} \times \frac{S_O}{K_{O,H}+S_O} - b \qquad \text{Equation 1.11}$$

Using this equation, the biomass growth rate can be predicted from only two variables, the substrate and oxygen concentration, since the other parameters remain constant for a given set of conditions. Examples of values used for modeling are q̂ = 3 g $COD_{removed}$ g^{-1} $COD_{biomass}$ d^{-1}, $K_{S,H}$ = 2 mg COD L^{-1}, $K_{O,H}$ = 0.2 mg O_2 L^{-1}, and b = 0.2 d^{-1} when the system is operated at a temperature of 20°C (Henze et al., 2000). Monod-like expressions can be introduced for all wastewater constituents that can become limiting for microbial growth, such as minerals, wastewater buffering capacity and inhibitory compounds. For example, the activated sludge model 3 (ASM3) uses Monod expressions for COD, ammonium, electron acceptors (oxygen, nitrate and/or nitrite), alkalinity and storage polymers

(Henze et al., 2000). Reaction rates, such as the maximum substrate uptake rate q̂ and the decay rate b, are dependent on temperature, according to the relationship (Metcalf & Eddy, 2003):

$$k_T = k_{20}\theta^{(T-20)} \qquad \text{Equation 1.12}$$

where k_T is the reaction rate at temperature T, k_{20} is the reported reaction rate at 20 °C, and θ is the temperature activity coefficient. This coefficient varies between 1.02 and 1.25. Between the range of 20 to 30 °C, a value of θ = 1.04 is often used. Due to the temperature dependency of reaction rates, temperature often has a strong influence on wastewater treatment processes, and may, for example, significantly impact the overall performance during cold seasons.

It should be noted that the above equations are instantaneous kinetic expressions and do not necessarily reflect equilibrium conditions over a prolonged period of time. After an initial start-up phase, activated sludge systems tend to evolve to a stable operation, which is called steady-state operation. During steady state, the rates of substrate removal, biomass growth, and decay are relatively constant, as are concentrations of biomass, residual substrate and minerals in the effluent. Since biomass levels remain constant, the specific biomass production rate equals the rate at which biomass leaves the system per unit of total biomass in the system:

$$\mu = \frac{biomass\ production\ rate}{biomass\ in\ system} = \frac{Q_w \times X_w + Q_e \times X_e}{V \times X} \qquad \text{Equation 1.13}$$

Where Q is the flow rate (L d^{-1}), X is the biomass concentration (mg COD L^{-1}), V is the reactor volume and subscripts w and e denote the waste stream and effluent stream, respectively, as can be seen in **Figure 1.1**.

At – and only at – steady-state conditions, and when no solids are entering the system through the influent, the inverse of the biomass production rate equals the solids retention time (SRT), i.e., the amount of time it takes for the body of sludge in the system to be renewed (Lawrence & McCarty, 1970). The SRT is a critical factor to consider when designing and operating a wastewater treatment, because of its strong influence on nearly all aspects of plant performance (Van Haandel & van der Lubbe, 2007). The longer sludge remains in the system, the longer it is subjected to endogenous decay and the more its growth will be offset by decay. The effect of a long SRT on lowering the net sludge production rate can be predicted using the non-biodegradable residue and the endogenous decay rate (Metcalf & Eddy, 2003):

$$Y_{h,obs} = Y_{h,max}\frac{(1+f_d \times b \times SRT)}{(1+b \times SRT)} \qquad \text{Equation 1.14}$$

Where $Y_{h,obs}$ is the observed (net) growth yield. The specific substrate uptake rate in steady-state conditions can be determined in function of the net amount of substrate the system removes per unit of time divided by the biomass level in the reactor. When the reactor volume is constant, the specific net biomass growth can be calculated from the specific substrate uptake rate and the observed yield. This gives the following equation:

$$\mu = \frac{1}{SRT} = \frac{Q_w \times X_w + Q_e \times X_e}{V \times X} = Y_{obs} \times \frac{(S_i - S_e) \times Q_i}{V \times X}$$ Equation 1.15

Where S_i and S_e are the influent and effluent substrate concentrations and Q_i is the influent flow rate.

3.2 Nitrogen removal

3.2.1 Nitrogen conversion reactions

Nearly all of the nitrogen in domestic wastewater is present in reduced form, either as dissolved ammonia (NH_4^+) or as organic nitrogen, which can be in dissolved or particulate form (Metcalf & Eddy, 2003). The conventional approach to nitrogen removal is biological treatment, which produces the harmless and non-reactive N_2 gas as the main end product. Biological nitrogen removal requires the concerted effort of autotrophic and heterotrophic microorganisms and revolves around the following processes:

- Hydrolytic release of ammonia during the degradation of organics by heterotrophs:

$$C_w H_x O_y N_z + \left(w + \frac{x}{4} - \frac{y}{2} - \frac{3z}{4}\right) O_2 \rightarrow wCO_2 + \left(\frac{x}{2} - \frac{5z}{2}\right) H_2O + zOH^- + zNH_4^+$$ Equation 1.16

- Nitritation, or the oxidation of ammonia to nitrite. This is the first part of nitrification, and is performed by autotrophs such as the aerobic ammonia oxidizing bacteria (AerAOB, e.g., *Nitrosomonas*) and ammonia oxidizing archaea (AOA):

$$NH_4^+ + \frac{3}{2} O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$ Equation 1.17

- Nitratation, or the oxidation of nitrite to nitrate. This is the second part of nitrification, and is performed by autotrophic nitrite oxidizing bacteria (NOB, e.g., *Nitrospira*, *Nitrobacter*):

$$NO_2^- + \frac{1}{2} O_2 \rightarrow NO_3^-$$ Equation 1.18

- Denitrification by heterotrophic bacteria, with nitrate or nitrite as electron acceptor (Equations 1.3 and 1.4)
- Anoxic ammonia oxidation (anammox) by the autotrophic anoxic ammonium oxidizing bacteria (AnAOB, e.g., *Brocadia*, *Kuenenia*):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$ Equation 1.19

Recently, two species of *Nitrospira* have been found to possess genes necessary for ammonia as well as nitrite oxidation (Daims et al., 2015; van Kessel et al., 2015). These species would thus be capable of completely oxidizing ammonia to nitrate, a reaction which is termed complete ammonia oxidation (comammox). It is unclear as to what extent comammox plays a role in wastewater treatment.

To accurately express the amount of oxygen needed to oxidize not only the organic matter but also the organic and ammonia nitrogen present in wastewater, the measure of total oxygen demand (TOD) is sometimes used, which can be calculated as TOD = COD + 4.57 N, where N is the concentration of organic and ammonia nitrogen (mg L^{-1}). Similar to the situation with heterotrophs, each of the above reaction results in biomass growth, and complete reaction balances may be determined for the processes of nitritation, nitratation, and anammox, taking the biomass yield into consideration (Barnes & Bliss, 1983; Matějů et al., 1992; Strous et al., 1998).

3.2.2 Plant configurations

Nitrogen removal technologies make use of a combination of these processes by sequentially treating the wastewater in aerated and non-aerated phases. It should be noted that activated sludge processes can be carried out with spatial separation of the different phases, e.g., in a series of continuous stirred-tank reactors (CSTR), or with temporal separation of the phases, e.g. in a sequencing batch reactor (SBR). In the descriptions below, the word 'phase' is used to stress that these plant configurations can be realized as part of a CSTR or an SBR design.

The most common technique is complete nitrification/denitrification (N/DN), whereby nitrogen is completely oxidized to nitrate (Equations 1.17 and 1.18) in aerobic conditions and subsequently reduced to N_2 (Equation 1.3) in anoxic conditions. N/DN treatment by means of an aerobic phase followed by an anoxic phase was first proposed by Wuhrman (1960) (**Figure 1.2 A**). This simple configuration is rarely used in practice, because it requires large amounts of oxygen in the aerated phases to completely oxidize COD to CO_2 and ammonia to nitrate, while the subsequent step requires addition of an external carbon source such as methanol to drive the denitrification reaction. In order to avoid the inefficiency of adding external carbon sources after oxidizing the COD that was present in the wastewater, N/DN plants often have a more efficient configuration that uses the pre-denitrification principle. First proposed by Ludzack and Ettinger (1962) (**Figure 1.2 B**), this configuration begins with an anoxic phase where the incoming COD is used to perform denitrification instead of being oxidized. Then follows an aerated phase where the reduced nitrogen from the influent is nitrified. The nitrate-rich mixed liquor is partially recycled to the anoxic phase and partially sent to the final clarifier. Because this configuration does not allow complete nitrogen removal, the Bardenpho process was proposed (Barnard, 1973) to combine the advantages of pre-denitrification (i.e., minimize the need for of external carbon sources) and post-denitrification (i.e., allow complete removal of residual nitrate prior to discharge) (**Figure 1.2 C**). However, even in WWTPs with an improved process configuration, methanol addition is often practiced during occasions when not enough bCOD is present to reduce the nitrate (De Clippeleir et al., 2013a). Stoichiometrically, 2.86 g COD is required to reduce 1 g of NO_3^--N (Equation 1.3). Taking into consideration that 20% of bCOD is always oxidized aerobically (Matějů et al., 1992), the bCOD/N ratio of wastewater needs to be higher than 3.4 g g^{-1} to allow N/DN.

To avoid the need for external carbon sources, especially if the wastewater has a low bCOD/N ratio, shortcut nitrogen removal processes have been developed. These avoid complete nitrification of

ammonium to nitrate. The first of these approaches is nitritation/denitritation (nit/denit), in which ammonium is oxidized to nitrite (Equation 1.17) and subsequently denitrified (Equation 1.4). Regardless of the configuration of the installation, the nit/denit process requires suppression of the NOB activity. This is an ongoing effort, but might be achieved by a combination of strategies involving intermittent aeration, aeration duration control, control of dissolved oxygen (DO) levels, and differential control of the SRT of AnAOB vs. other species (Seuntjens et al., 2016). Stoichiometrically, the nit/denit approach requires 1.71 g bCOD to reduce 1 g of NO_2^--N. With consideration of 20% aerobic bCOD oxidation, this requires a minimum bCOD/N ratio of the wastewater of 2.1 g g^{-1}. In case wastewaters have even lower bCOD/N ratios, for example after a primary treatment stage where much of the bCOD is removed but not the nitrogen, partial nitritation/anammox (PN/A) may be the preferred approach. In this approach, a part of the nitrogen is oxidized to nitrite (Equation 1.17) while another part remains in reduced form. Subsequently, nitrite and ammonium are combined in the anoxic phase to form nitrogen gas (Equation 1.19). The PN/A approach does not require a carbon source and could theoretically be applied to treat wastewaters with a bCOD/N ratio down to 0. Like in nit/denit, PN/A requires the suppression of NOB. The PN/A process has been successfully applied to wastewaters with temperatures above 25°C and nitrogen concentrations above 100 mg L^{-1}, such as the relatively warm and nitrogen-rich reject water that is produced after anaerobic digestion (AD) of sludge in the WWTP's side stream. Direct PN/A treatment of mainstream wastewater has not been fully developed.

Biological nitrogen removal is a non-renewable process, releasing nitrogen in the atmosphere as N_2 gas. Alternative physical methods have been developed that allow recovery of nitrogen as mineral fertilizer, for example by ammonia stripping and recovery as ammonium sulfate (Menkveld & Broeders, 2015), precipitation as magnesium ammonium phosphate (struvite) (Doyle & Parsons, 2002), or regenerative adsorption / desorption using zeolites (Karapınar, 2009; Widiastuti et al., 2011). This fits the philosophy of redesigning the water cycle to accommodate resource recovery (see section 2). Nitrogen recovery from concentrates sources such as source-separated urine or WWTP side streams may be cost-efficient (Menkveld & Broeders, 2015; Mulder, 2003), while biological nitrogen removal is still the preferred technology in mainstream wastewater treatment (Courtens, 2015). Finally, potent greenhouse gases such as NO and N_2O are produced during the various steps of biological nitrogen removal (Colliver & Stephenson, 2000). Although rarely monitored, greenhouse gas emissions from inefficient biological nitrogen removal processes can account for up to 80% of a WWTP's operational emissions of CO_2-equivalents (Desloover et al., 2012b) and have a critical influence on the overall sustainability of the anthropogenic water cycle.

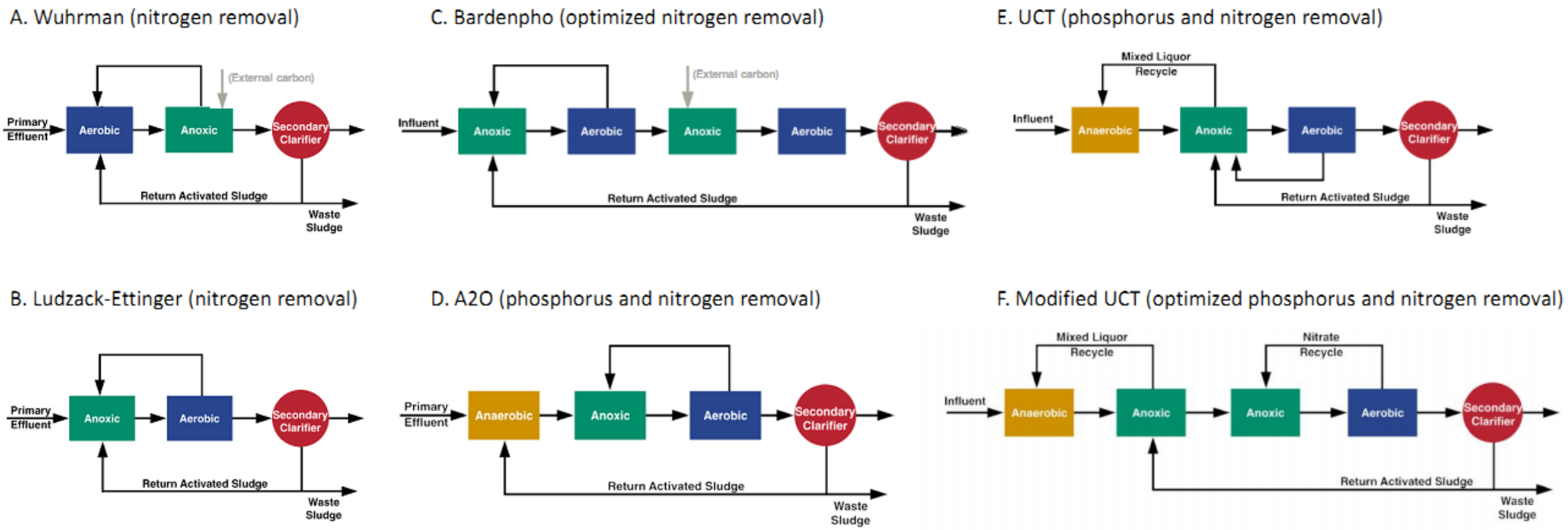


Figure 1.2: Different process configurations for biological nitrogen and phosphorus removal. A: Wuhrman process; B: Ludzack-Ettinger process; C: Bardenpho process; D: Anaerobic-anoxic-oxic process; E: University of Cape Town process; and F: Modified University of Cape Town process. Grey arrows indicate possible dosage of external carbon source. Figure redrafted after Grissop (2010).

3.3 Phosphorus removal

Removal of phosphorus from wastewater can either occur by chemical precipitation or by biological phosphorus removal. In chemical phosphorus removal, dissolved orthophosphate (PO_4^{3-}) is precipitated out of the wastewater by adding Fe^{2+}, Fe^{3+} or Al^{3+}-based salts as part of the primary, secondary or even tertiary treatment stage, after which insoluble metal-phosphate salts are formed. While chemical phosphorus removal does not require large capital investments, operational costs for the addition of chemicals can be high (Rybicki, 1997). Furthermore, the iron and aluminum salts may have an inhibitory effect on biogas production during anaerobic digestion of the waste sludge (Cabirol et al., 2003; Gossett et al., 1978) and their added weight increase the cost of sludge disposal.

Biological removal technologies do not require addition of chemicals, but requires a more complex plant configuration, which results in higher costs for investment, maintenance and control (Jiang et al., 2005). Enhanced biological phosphorus removal (EBPR) makes use of the metabolism of phosphorus accumulating organisms (PAOs), which are capable of storing large quantities of phosphorus in their cells as polyphosphate. In anaerobic conditions, PAOs use these polyphosphate reserves as energy source for the uptake and storage of simple carbon sources such as volatile fatty acids (VFAs). In anoxic or aerobic conditions, the PAOs oxidize their stored carbon sources with nitrate or oxygen as electron source, respectively, and use part of their energy to take up phosphorus again. Net removal of phosphorus can be achieved by wasting the sludge after the anoxic or aerobic phase. Since ordinary heterotrophic organisms cannot take up large quantities of VFAs in anaerobic conditions, selective enrichment of PAOs can be achieved by starting the wastewater treatment train with an anaerobic phase.

The simplest process configuration to achieve EBPR is the A/O process or Phoredox process, in which an anaerobic phase is followed by an aerobic (oxic) phase. Because this configuration does not allow efficient nitrogen removal, a combination of the Ludzack-Ettinger and the A/O process – the anaerobic-anoxic-oxic (A2O) process – may be preferred (**Figure 1.2 D**), in which EBPR is combined with biological nitrogen removal. Several modifications and additions to these basic configurations have been proposed to further improve process efficiency. For example, the University of Cape Town (UCT) process is a modification of the A2O process that prevents nitrate coming into the anaerobic zone via the return sludge (**Figure 1.2 E**), while the modified UCT configuration further improves process control by separating the anoxic phase into two zones (**Figure 1.2 F**). In the modified UCT configuration, the different return flows can be controlled in such a way that incoming VFAs are preferentially used for phosphorus removal before any denitrification can occur. The presence of nitrate in the anaerobic phase should be avoided, where it would partially erase the selective advantage of PAOs over denitrifying heterotrophs.

Similar to the case for nitrogen, recovery of phosphorus during side stream treatment is currently under development. For example, phosphorus in digested sludge supernatant can be precipitated as struvite by adding magnesium salts (Doyle & Parsons, 2002; Shu et al., 2006) or regenerated through adsorption and subsequent desorption using zeolites (Karapınar, 2009).

4 Microbial ecology of activated sludge

4.1 Approaches to studying the microbial community

Activated sludge consists of a dense consortium of several thousand species of bacteria, together with archaea, algae, fungi, protozoa and small metazoa (Metcalf & Eddy, 2003). The rich community of activated sludge can be studied on various levels. First, the community can be described by itself, with respect to:

- species composition,
- community richness (i.e., the number of species, genera, families, orders, classes or phyla),
- community evenness (i.e., the equality of the abundance distribution across species),
- community diversity (i.e., richness corrected for evenness),
- community dynamics (i.e., temporal variability),
- community interactions (i.e., co-occurrence or co-exclusion),

and so on. In some studies, the sludge community is considered to be the sum of its members, and a focus is laid on studying the metabolic capabilities of individual species or genomes. A prime example of a database that catalogues such information is the MIDAS field guide (McIlroy et al., 2015). However, the complex, rich and dynamic nature of activated sludge communities makes it nearly impossible to describe the ecology of each of the several thousand individual members, and attempts to do so are of questionable relevance. Therefore, species-based research should be complemented with research on community-wide interactions. By studying interaction networks, it can be understood in what way the activated sludge processes is influenced by interactions such as symbiotic relationships (e.g., syntrophy), competition and predation. Keystone members of the community, i.e., members that have a disproportionate influence on the community relative to their abundance, can be identified from co-occurrence networks (Berry & Widder, 2014; Roume et al., 2015).

Second, the community can be studied in terms of functional output, such as substrate removal, biomass growth, or end product formation. This can be done both on the level of species (e.g., metabolic capacities) or the community level (e.g., trophic interactions). Functional studies may focus on individual species, but often group micro-organisms according to their effect on the sludge morphology, e.g., filaments bacteria, foaming bacteria and floc formers, or according to their metabolism, e.g., heterotrophs, denitrifiers, AOB, NOB and PAOs. Functional groups may comprise many species that play similar ecological roles and therefore bear a certain functional redundancy. Much of the research and development of activated sludge systems is performed at the level of functional groups

Third, the influence of operational (e.g., oxygen levels, reaction times, feeding pattern) and environmental factors (e.g., temperature, influent composition, temporal shocks) on the community structure and function can be studied. That way, the processes that influence the community can be

better understood, and strategies can be developed to better control functional output. Ideally, the microbial community should be studied simultaneously on all three levels. Despite improved process control, many wastewater treatment plants (WWTP) still struggle with operational problems related with a perturbance of the microbial community, and the need remains for a better knowledge of the activated sludge community in relation to its structure and dynamics, its functional output and its sensitivity toward environmental and operational parameters.

4.2 Progress in ecological research on activated sludge

Throughout much of the 20th century, activated sludge communities have been studied in the traditional microbiological approach, which focused on species isolation, cultivation, and identification by their morphology, metabolic capacities, and staining properties. However, these traditional methods were insufficient to describe the complete activated sludge microbiome, because of its vast richness and high degree of functional redundancy, combined with the fact that natural ecosystems typically have a large fraction of species that cannot be cultured in the lab (Trevors, 2011). Only with the rise of high-throughput molecular techniques and metagenome sequencing, have researchers been able to describe and study the nearly complete microbial community.

Since the rise of high-throughput sequencing technologies, researchers have been able to monitor the community dynamics of activated sludge over relatively long terms and explore the interactions of species with environmental factors, other species and functional output of the ecosystem, on a relatively large scale with limited efforts and costs. A growing body of scientific literature has embraced genome-based detection techniques to identify the functional role of certain microorganisms in processes such as nitrogen removal (Juretschko et al., 1998), phosphorus removal (Crocetti et al., 2000), and floc formation and floc stability (Rosselló-Mora et al., 1995; Wilén et al., 2008). Environmental parameters that have been correlated with changes in the community structure include the temperature, SRT, sludge-specific loading rate (SLR), substrate concentrations, inorganic nitrogen concentrations, and dissolved oxygen (Hai et al., 2014; Ibarbalz et al., 2014; Valentin-Vargas et al., 2012; Wells et al., 2011). Bacterial species from the same phylum or class were shown to co-occur more frequently than expected by chance (Ju et al., 2014), while co-exclusion occur primarily between taxonomically distant species (Ju & Zhang, 2014). The community diversity, evenness and dynamics, measured as species turnover rate or similarity decay of samples over time, were all shown to have a positive influence on ecosystem function and resilience (Briones & Raskin, 2003; Johnson et al., 2014; Saikaly & Oerther, 2011; Wittebolle et al., 2009). The current state-of-the-art of activated sludge community research revolves around description of taxonomic richness, functional richness, and dynamics of the community under the influence of environmental or operational factors. In order to develop advanced strategies to control the activated sludge process on a community level, much more progress needs to be made in the understanding of how communities operate, change, and interact with their environment.

High-rate activated sludge (HRAS) communities (see section 5.3) are thought to differ from conventional communities, but are less studied. Early research on the microbial ecology of HRAS systems led to the observation that, while low-rate systems had a rich community of bacteria, protozoa and metazoa, HRAS systems only supported growth of the fastest-growing bacteria (Böhnke et al., 1997c; Schürmann, 1984). Growth of protozoa such as flagellates and ciliates was considered undesirable, because their predation on the sludge bacteria would increase the overall oxygen demand without improving COD removal from the influent (Böhnke et al., 1997a). Research on the microbial ecology of high-rate activated sludge has remained scarce, and not until the past few years has a number of studies started to fill the knowledge gap (for example, Faust et al., 2015; Gonzalez-Martinez et al., 2016; Teksoy Başaran et al., 2014; Valentin-Vargas et al., 2012).

4.3 Composition of sludge flocs

Floc-forming bacteria are responsible for the structural integrity of activated sludge flocs and are therefore of critical importance for processes such as clarification of the effluent, retention of a viable sludge mass in the system, and sludge dewatering. A well-settling, dense floc, however, consists both of floc-forming and filamentous bacteria. A few filaments form a structural backbone in the center of the floc, which is then surrounded by a mass of floc-forming organisms (Jenkins et al., 2004). As such, both the excessive growth of filaments as well as floc formers may cause problematic sludge settling. Excessive growth of floc fragments may cause pin-point sludge and slime formation, while excessive protrusion of filamentous bacteria may cause foaming and bulking (i.e., floating or badly settling) sludge (**Figure 1.3**).

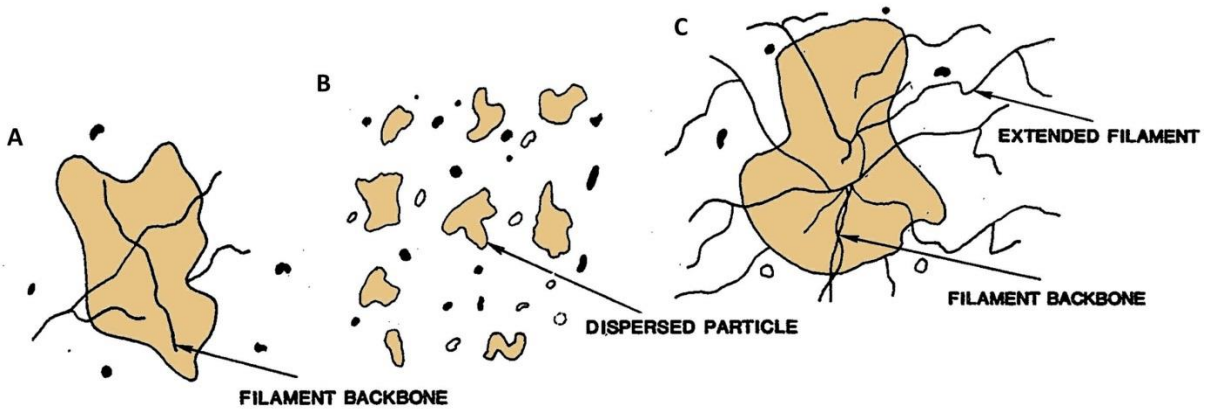


Figure 1.3: Morphology of (A) normal, (B) pin-point, and (C) filamentous bulking sludge flocs. After Jenkins et al. (2004).

Of all floc-forming bacteria, perhaps the genus *Zoogloea* is best known, although floc formation has also been linked to the presence of other genera, such as *Achromobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium* and *Pseudomonas* (McKinney, 2004). As the name '*Zoogloea*' (Greek for 'living glue') aptly recalls, the ability to grow in flocculent form lies in the formation of adhesive carbohydrates outside the cell wall. These carbohydrates are part of the layer of extracellular polymeric substances

(EPS) that surrounds cells and is responsible for the surface properties of activated sludge bacteria (Sheng et al., 2010). A distinction should be made between flocculent growth (i.e., division and growth of *Zoogloea*-like cells within a carbohydrate matrix) and bioflocculation (i.e., the adhesion of previously unattached cells and/or particles), although in both processes the adhesion is mediated by the EPS surface (Dugan et al., 2006).

5 Technological strategies for COD recovery

5.1 Bioflocculation

5.1.1 Bioflocculation mechanisms

Bioflocculation is the process of physical adhesion between small sludge flocs and other organic particles, to form larger flocs that can be separated from the liquid by means of settling, membrane filtering or other processes. Surface adsorption of particulate organics onto the sludge flocs by the mechanism of bioflocculation plays an important role in the activated sludge process, because hydrolytic enzymes present in the floc surface can help degrade particulate organics (Frølund et al.; Zhang et al., 2014), and because sorption of particulate substrate onto sludge flocs without immediate oxidation is one of the mechanisms that allows recovery of organics from sewage. There is still uncertainty about the relative importance of different physical adhesion mechanisms in the bioflocculation process. As reviewed by Sobeck and Higgins (2002), these are (1) the Derjaguin-Landau-Verwey-Overbeek (DLVO) mechanism, in which bioflocculation is the result of the counteracting forces of van der Waals-attraction and electrostatic repulsion due to the electric double layer around organic particles, whose EPS layer is predominantly negatively charged, (2) the divalent cation bridging (DCB) mechanism, in which divalent cations such as Ca^{2+} and Mg^{2+} act as bridging agents between the negatively charged moieties of the EPS layer, and (3) the alginate mechanism – a subset of the DCB theory – in which aggregation of alginates in the EPS, under the influence of Ca^{2+}, is the main mechanism of bioflocculation.

The EPS layer must, however, not simply be regarded as a homogenous mass that is negatively charged. Its composition varies according to the specific growth conditions, but its main components are carbohydrates and proteins, followed by humic substances, nucleic acids and lipids (Sheng et al., 2010). Proteins are often cited as the most important contributors to the bioflocculation ability of sludge (Wilén et al., 2003; Xie et al., 2010) because their positively charged, negatively charged and hydrophobic functional groups can engage in versatile interactions with other particles, although it has been argued that carbohydrates also contribute to bioflocculation due to the presence of hydrophilic and hydrophobic regions (More et al., 2014). The relative ratio of (negatively charged) carbohydrates and (variable) proteins was found to be a stronger predictor of surface properties and bioflocculation capacity of sludge than their absolute quantities (Liao et al., 2001).

A clear distinction should be made between the bioflocculation process itself, and characteristics that are affected by the bioflocculation behavior of the sludge, such as sludge density, settleability and dewaterability. While the quality of the EPS – i.e., its hydrophobicity and surface charge – may positively affect the bioflocculation affinity of sludge, high quantities of EPS may be associated with bad sludge settleability and compressibility (Liao et al., 2001; Wilén et al., 2003). An abundant presence of EPS may be associated with viscous bulking, when the production of slime creates low-density sludge that can wash out with the effluent (Jenkins et al., 2004). Studies report that the production and composition of EPS are influenced by operational factors, such as substrate type, nutrient availability, SRT, presence of ions and heavy metals, shear rate and oxygen availability (Sheng et al., 2010, and references therein). In sludge granules, which depend on an EPS matrix for their structural integrity, the microbial community shows a layered distribution (Vlaeminck et al., 2010), and shifts in microbial community have been observed during the granulation process (Ding et al., 2015b; Etchebehere et al., 2003). This suggests that the quantity and quality of EPS may be associated with the structure of the microbial community; a fact which has been supported by metagenomic analysis. For example, Albertsen et al. (2013) demonstrated that alginate production genes were mainly associated with the phylum Bacteroidetes. It seems likely that shifts in microbial community structure may influence the production of EPS and affect its functionality towards the bioflocculation process.

5.1.2 Improving the bioflocculation capacity

Given its importance in the activated sludge process, several strategies have been proposed to improve the bioflocculation capacity of sludge. Because these strategies also have an effect on the intracellular storage of substrate in the form of poly-β-hydroxybutyrate (PHB) or other polyhydroxyalkanoates (PHAs), surface adsorption and storage are often grouped together under the term 'biosorption' (see Glossary), denoting the collective uptake of organic substrate by means of surface *ad*sorption and intracellular accumulation and storage (i.e., *ab*sorption) (Majone et al., 1999). Storage of PHAs has been linked to an improved settleability of sludge flocs (Gerardi, 2003; Oshiki et al., 2010) and is a main mechanism of interest, next to adsorption, that allows recovery of organics from sewage. For these reasons, adsorption and storage will often be considered together in this work.

To improve biosorption, strategies have been developed to favor growth of floc-forming bacteria over filaments. This is done by the principle of kinetic selection, first proposed by Chudoba et al. (1973), also known as the accumulation-regeneration theory. The main assumption of kinetic selection is that filamentous bacteria are better capable than floc-formers to grow at low substrate concentrations, because they have a higher substrate affinity (lower $K_{S,H}$, Equation 1.10). Floc-forming bacteria, on the other hand, are better capable of fast uptake of substrate (high $\hat{q}$, Equation 1.10) and storage, which makes them more resistant to subsequent starvation. Therefore, while it is meant as a strategy to improve floc formation and settling, what kinetic selection really does is promote the growth of bacteria capable of fast growth under high SLR conditions, as well as select for substrate storage and/or adsorption. Cech et al. (1985) determined kinetic constants for a

conventional activated sludge system and a selector system, and determined that the $\hat{q}$ for valeric acid, glutamic acid and tyrosine were 35, 42 and 40 $\times 10^{-3}$ h^{-1} in the CAS system and 99, 106 and 55 $\times 10^{-3}$ h^{-1} in the selector system, respectively, and that $K_{S,H}$ was 1.18, 0.56 and 0.91 mg L^{-1} in the CAS system and 3.20, 3.71 and 3.66 mg L^{-1} in the selector system, respectively. The increase in $\hat{q}$ and $K_{S,H}$ from the CAS to the selector system may have been caused by higher occurrence of floc-formers in the selector system. It should be noted that, while attempts to improve settleability by means of kinetic selection may be successful, the kinetic difference between floc-formers and filaments is not absolute. In many cases of filamentous bulking, the majority of the sludge mass still consists of actively growing floc-formers, while some filamentous bacteria have been shown capable of substrate storage (Beccari et al., 1998; Martins et al., 2003b).

In practice, kinetic selection of floc-forming bacteria can be achieved by subjecting the sludge to alternating high and low substrate availability. This is called a feast-famine regime or a substrate gradient, and is the basis of several process strategies designed to improve sludge settleability. A first strategy consists of adding an initial contact zone or 'selector' before the main treatment, either as a separate tank or as a sectioned part of the main basin, where return sludge is mixed with the incoming influent (Chudoba et al., 1973). Because the ratio of substrate to sludge (food-to-microorganism or F/M ratio) is high in the selector and low in the main treatment basin, an effective feast-famine regime is created. Selector processes are popular because they can be retrofitted to existing installations with a limited aerial footprint. Selectors can be aerated or non-aerated, but this choice may impact their performance (Martins et al., 2003a). Implementation of selector processes does not always have successful results, and their performance depends on conditions such as the strength of the feast-famine regime, basin volumes and suspended solids concentrations (Gray et al., 2006). Ironically, the strategy of creating a substrate ratio to improve sludge settleability can sometimes be the cause of sludge bulking. When the contact between sludge and substrate during the high F/M phase lasts too long, rapid growth of *Zoogloea*-type bacteria can occur, which causes viscous bulking (Martínez i Puentes, 2006).

A second strategy to obtain kinetic selection is to approach plug-flow conditions. Plug flow is a situation where a body of water flows along a channel without longitudinal mixing. If wastewater and return sludge would flow along a narrow channel in ideal plug flow conditions, the substrate would decrease along the way as it was consumed by the bacteria, creating a natural substrate gradient. However, ideal plug flow is impractical to achieve, because of the need of a long and narrow treatment channel, and has the disadvantage of not allowing dilution of the influent to protect the sludge from peak loads or toxic pulses (Metcalf & Eddy, 2003). Wastewater treatment plants are often equipped with reactors that have intermediate characteristics between ideal CSTR and ideal plug flow. For example, the plug-flow character of an activated sludge basin can be increased by installing baffles in the treatment basin to prevent complete mixing and direct the flow into a channel-like pattern, or a treatment plant can implement staged treatment where, instead of a single large CSTR basin, a limited number of smaller CSTR reactors are coupled in a cascade series.

A third strategy for kinetic selection is the contact-stabilization configuration. Proposed as early as the 1920s (Coombs, 1921), contact stabilization was designed to employ biosorption for the treatment of wastewaters with a high fraction of colloidal organics that do not settle by themselves (Benefield & Randall, 1976). The principle of contact stabilization is that after settling, return sludge is aerated in a stabilization phase to promote biomass growth on stored and adsorbed substrates. Subsequently, it is mixed with influent in an aerated or non-aerated contact phase to allow, besides regular growth, adsorption and storage of new substrates (**Figure 1.4**). This creates a feast-famine cycle in which the aeration of the return sludge allows regeneration of its sorption and storage capacity (Vásquez-Sarria et al., 2011). The contact stabilization process has been described under various names, including the biosorption, the dual aeration, the sludge reaeration, the bioflocculation, the bio-flocculation-adsorption, and the contact-adsorption-regeneration-stabilization process (Khararjian & Sherrard, 1978; Liu et al., 2009; Zhao et al., 2000).

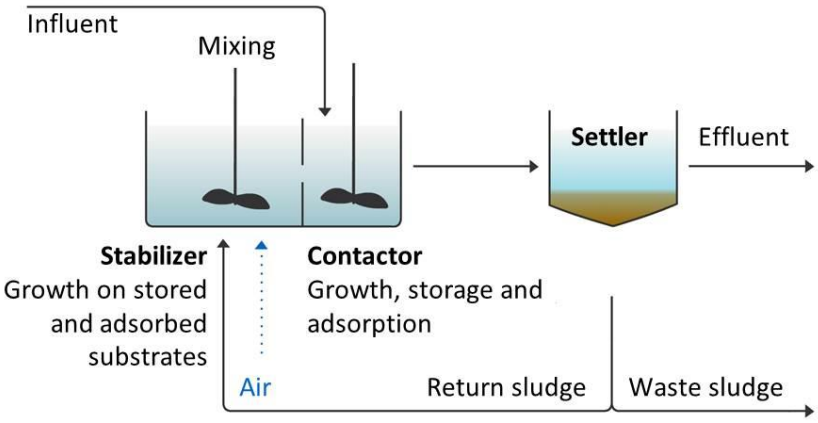


Figure 1.4: Schematic representation of a basic contact stabilization process, after Meerburg et al. (2015).

Contact stabilization plants were popular in the second half of the 20th century because they required smaller volumes compared to CAS plants (Ramalho, 1977b). In a conventional execution, the tank volumes of the contact stabilization process were chosen such that the hydraulic retention time (HRT) of the contactor was between 0.5 and 3 hours, and the HRT of the stabilizer between 3 and 6 h (Khararjian & Sherrard, 1977). The contact stabilization process was also claimed to produce less waste sludge, because the system configuration allow independent control over microbial growth and decay (Orhon, 2015, and references therein). Due to the short contact times and quick passage of wastewater through the system, the effluent of quality of contact stabilization systems was sensitive to fluctuations of the influent load. Moreover, requirements for biological nitrogen and phosphorus removal were incompatible with the contact stabilization design. For these reasons, many plants originally built as contact stabilization systems were converted to CAS plants (Orhon, 2015; Rittmann & McCarty, 2001). Apart from the contact stabilization process, other processes designed to improve sludge settleability made use of aeration of (part of) the return sludge, such as

the Hatfield process (Hatfield, 1959) and the Kraus process (Kraus, 1945). To facilitate biological nitrogen removal in the contact stabilization configuration, hybrid processes such as the regeneration-denitrification-nitrification (RDN) process have been proposed (Kos et al., 1992).

5.2 HRT and SRT as control tools

The hydraulic retention time (HRT) is the average time that water spends in the system, and is calculated by dividing the system volume by the flow rate of incoming water. Because most activated sludge systems have an incoming stream of influent as well as return sludge, it is good practice to distinguish between nominal and actual HRT. The nominal HRT (HRT_{nom}) is the average time that the influent will spend in the system, which does not depend on the return sludge flow rate. The HRT_{nom} is the most common of both parameters and is often simply reported as the 'HRT'. The actual HRT (HRT_{act}) represents the time spent by the mixed liquor suspended solids (MLSS) in the system before it is sent to the settlers and partially recycled. It is also called contact time or reaction time.

$$HRT_{nom} = \frac{V}{Q_i} \quad ; \quad HRT_{act} = \frac{V}{(Q_i+Q_r)} \qquad \text{Equation 1.20}$$

Where Q_r is the flow rate of the sludge return. The HRT of a system can be changed by providing a larger or smaller reactor volume. Eliminating the need for constructing large, expensive reactor basins, was one of the reasons why high-rate activated sludge processes were developed (see section 5.3). The HRT can be an important control parameter that determines the extent to which slow reactions can take place. For example, processes of a physicochemical nature such as biosorption are completed in the order of minutes (Bunch & Griffin, 1987; Guellil et al., 2001; Wahlberg et al., 1994), while biological mechanisms such as hydrolysis, oxidation and cell growth take longer. As the HRT lengthens, BOD and COD removal efficiencies typically increase (Barr et al., 1996). During the course of organics removal, the easily degradable substrates are degraded first and the microorganisms gradually move towards the more difficult compounds. This phenomenon can be exploited for the removal of certain recalcitrant compounds and micropollutants, by operating the system at very long HRTs (Petrie et al., 2014).

With respect to the solids retention time (SRT), the simplest way to regulate the SRT of an activated sludge process is by adjusting the sludge wasting rate Q_W (Equation 1.15). A lower sludge wasting rate means that the sludge is replaced slower in the system and that the SRT becomes longer. Microorganisms can remain in the system when their growth rate is higher than the rate at which the sludge is renewed. For a given species of microorganism, the minimum SRT to avoid 'washout' from the system can be approximated by the following equation (Abeysinghe et al., 2002):

$$\frac{1}{SRT^{min}} = Y_{sp,max} \times q_{sp,max} \times \frac{S_{lim}}{K_{sp,lim}+S_{lim}} - b_{sp} \qquad \text{Equation 1.21}$$

Where the subscript sp denotes the parameters for the specific species, and the subscript lim denotes the limiting substrate. At short SRTs, only the fastest growing bacteria can remain in the system, while at longer SRT, the system can harbor a rich microbial community containing slow-

growing bacteria, archaea and eukaryotes. Indeed, systems with short SRTs have a less diverse microbial community than systems with longer SRTs (Duan et al., 2009; Gonzalez-Martinez et al., 2016). Bacterial grazing by protozoa and other eukaryotes can significantly impact the sludge community and performance at longer SRTs (Kaewpipat & Grady, 2002) and is one of the main contributors to endogenous decay of the sludge mass over time (Van Loosdrecht & Henze, 1999). Accordingly, at longer SRTs, the fraction of active bacterial cells decreases as the sludge is progressively composed of inactivated and decayed biomass (Ramalho, 1977a; Van Haandel & van der Lubbe, 2007). CAS systems are typically operated at SRTs from 10 to over 30 days, firstly because long SRTs will cause a low net sludge production and decrease the costs for sludge disposal in case the sludge is not used for on-site energy recovery – costs for sludge handling and disposal may run up to 40% of a plant's total operational expenditure (OPEX) (Zessner et al., 2010). Secondly, too low an SRT would result in deteriorating removal efficiencies of organic matter and nutrients, because the metabolic capacities of the sludge community become limited when the diversity drops (Haider et al., 2003; Rittmann, 1996). For example, the minimum required SRT for complete nitrification at a temperature of 14°C is around 7.5 d under optimal conditions of oxygen and ammonium concentrations (Henze et al., 2008), which imposes an absolute lower limit on the SRT in case biological nitrogen removal needs to be achieved (see section 3.2). Biological removal of phosphorus requires an SRT above 8-15 d (Mulkerrins et al., 2004), although P removal has been successful at SRTs as low as 2 d (Ge et al., 2015). The downside of operating systems at a long SRT is the high degree of endogenous decay, which results in relatively high aeration requirements and severely limits the amount of energy that can be recovered from the waste sludge. The relationship between SRT and oxygen demand can be visualized in a theoretical example (**Figure 1.5**), showing that that the amount of COD removed by direct exogenous respiration is independent of the SRT, but the net sludge production decreases with SRT. The total aeration requirement increases with SRT, due to the higher fraction of sludge lost through endogenous respiration. Because of the trade-off between sludge production and aeration requirement, a plant that seeks to minimize sludge production, e.g., to lower the disposal costs when no on-site AD can be performed, will face higher aeration costs.

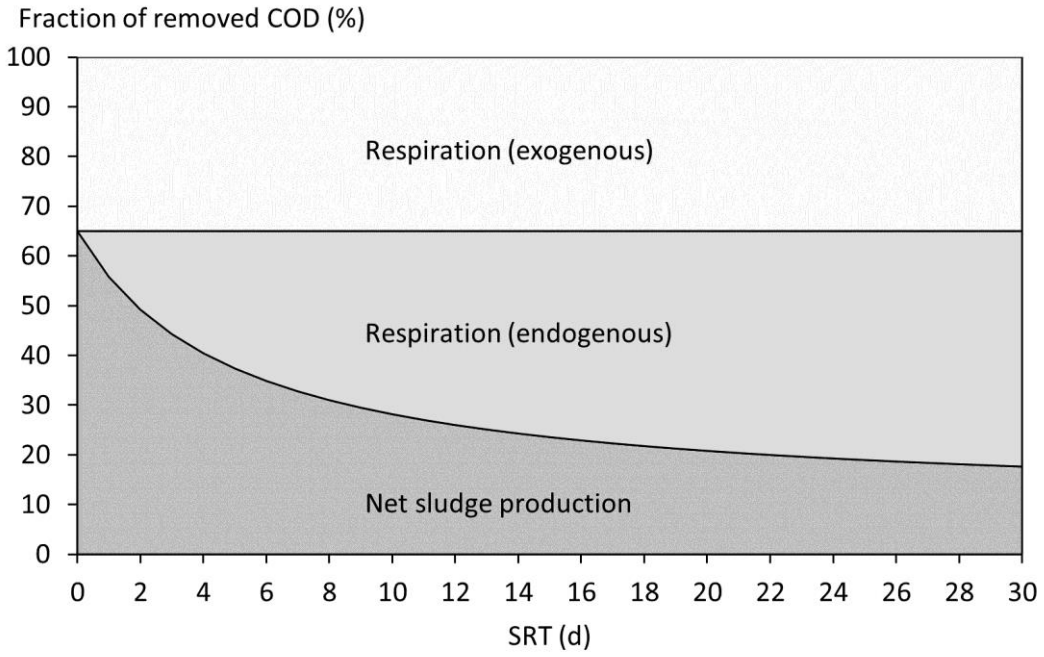


Figure 1.5: Fractionation of removed COD in function of SRT, showing the fractions of COD removed by direct exogenous respiration (i.e., 1 - Y_{max}), by net sludge production (i.e., Y_{obs}), and by indirect respiration due to endogenous decay of produced sludge (i.e., Y_{max} - Y_{obs}), in function of the SRT. The Y_{max} was assumed to be 0.65 g COD_{sludge} g^{-1} $COD_{removed}$, and Y_{obs} was calculated according to Equation 1.14, where F_b was 0.15 and b was 0.2 d^{-1}.

To increase the COD recovery potential of a wastewater treatment system, it can be operated at a short HRT (to reduce the extent of substrate oxidation) and a short SRT (to reduce the extent of sludge oxidation). This is the basic principle of high-rate activated sludge processes (see section 5.3), together with the application of a high SLR.

5.3 High-rate activated sludge

High-rate activated sludge (HRAS) processes are characterized by high sludge-specific loading rates (SLR) and short SRTs. A strict definition does not exist, but systems with an SLR above 2 g bCOD g^{-1} VSS d^{-1} and an SRT of around or below 2 d are considered high-rate processes. By comparison, a low-rate process is often defined as having an SLR below 0.6 g bCOD g^{-1} VSS d^{-1} and an SRT above 3 d (Metcalf & Eddy, 2003). Whether a system with intermediate characteristics is considered high-rate depends on the author. Conventional activated sludge processes typically operate well within the low-rate ranges, with values for SLR and SRT around 0.25 g bCOD g^{-1} VSS d^{-1} and >10 d, respectively. Additionally, HRAS processes typically operate at a shorter HRT, in the order of minutes to a few hours, compared to conventional processes, whose HRT range from the order of hours to even days.

The use of high-rate processes for the purpose of wastewater treatment has been proposed as early as the 1920s (Buswell & Long, 1923, cited by Jimenez et al., 2015). HRAS processes became popular

as stand-alone treatment technologies because they achieved an organics removal efficiency that was considered acceptable – 80-85% BOD removal – with a considerably lower aerial footprint compared to CAS treatment (Wuhrmann, 1954). Stand-alone HRAS systems continue to be used for wastewater treatment in the United States until today (DeArmond et al., 2015; Jimenez et al., 2015). In Europe, HRAS technology would primarily catch on as part of two-staged wastewater treatment. In response to tightening effluent discharge standards for BOD, Böhnke (1977) developed the Adsorptions-Belebungsverfahren or AB-process, a two-stage process composed of a HRAS-system and a conventional low-rate system. In this context, high-rate activated sludge treatment is sometimes referred to as the A-stage, while low-rate treatment is referred to as the B-stage. True to the definition of a two-stage system (Imhoff, 1955), the two stages of the AB-system operate with separate sludge settling and recycle (**Figure 1.6**).

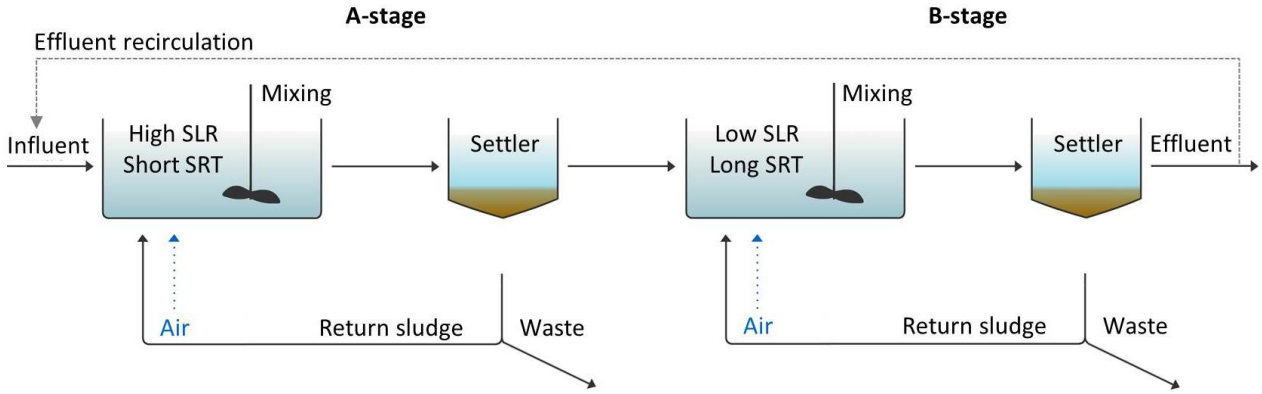


Figure 1.6: Schematic representation of a traditional AB-system.

The AB-system was originally designed to remove BOD from wastewater at lower operational costs, energy requirement and aerial footprint compared to CAS treatment, while it would allow for a higher biogas production from anaerobic digestion of the sludge (Böhnke, 1984; Versprille et al., 1985). Because the A-stage may act as a buffer for the B-stage, a two-stage system would also be more resistant towards pH and toxicity shocks (Böhnke, 1985). After the original plant in Krefeld, Germany, proved successful, many more AB-plants were built in Germany and elsewhere in Europe (Gethke, 1984; Salomé, 1990). Later, as nutrients became an important criterion in the increasingly strict effluent standards, it was realized that the B-stage needed to be redesigned to accommodate nitrogen and phosphorus removal, should the AB-system remain an adequate technology for wastewater treatment (Salomé, 1990). Depending on the specific type of wastewater, BOD and COD removal in the A-stage could be as high as 80%, but the A-stage effluent needed to contain a sufficient amount of BOD to allow denitrification in the B-stage (Böhnke et al., 1997b). Due to continued difficulties of some plants to achieve a sufficient effluent quality, many original AB-installations were converted to conventional systems (de Graaff & Roest, 2012, personal communication with plant operators). Plants that still operate according to a traditional AB-system often need to recirculate a fraction of the final effluent, containing nitrate that was insufficiently

removed, back to the A-stage to allow denitrification. While effluent recirculation can improve the overall removal of nitrogen, it increases the hydraulic load on the plant, which results in lower actual HRTs and may increase the risk of sludge washout from the settlers.

Up to 60% of the COD removal in HRAS systems is attributable to physicochemical mechanisms such as bioflocculation and settling of particulate organics (Wuhrmann, 1954), while oxidation of COD accounts for 12-50% of total COD removal (Ge et al., 2013; Haider, 2002; Haider et al., 2000). This depends on conditions such as the SRT and HRT: a shorter SRT results in lower losses of sludge mass through endogenous respiration, while a shorter HRT limits the extent of degradation of particulate and dissolved substrate that is removed through adsorption and/or storage but not yet degraded (see section 5.2). Because of these low oxidation percentages, HRAS systems have a high production rate of young sludge that is easily convertible to biogas (Ge et al., 2013; Trzcinski et al., 2016b). As a rule of thumb, in primary sludges, about 60% of the COD content can be digested, while for secondary sludges, this is only 30% (Van Haandel & van der Lubbe, 2007). Because of their high COD recovery and digestibility potential, the interest in HRAS systems has recently revived in the context of resource recovery from wastewater. High-rate activated sludge technology plays a central role in process treatment schemes designed to recover organic matter and energy from wastewater. While once viewed as old-fashioned by some, AB-installations may well be the plants that are closest to achieving energy self-sufficiency, when given a few modifications (Wett et al., 2007). **Table 1.1** provides a non-exhaustive list of wastewater treatment plants with an AB-type design that are currently still in operation.

Table 1.1: WWTPs with a configuration based on the AB-system. The table is non-exhaustive and not all plants are confirmed to be currently operational as an AB-system. Only domestic and combined industrial/domestic WWTPs were included. Fifteen more AB-plants reported in literature were confirmed to have converted to other configurations through personal communication, and were not taken up in the table. CEPT: chemically enhanced primary treatment. MBBR: moving bed biofilm reactor. n.a.: not applicable or no information available. PE: population equivalents. [a]The Hybrid© process is an AB-process with sludge recycle from the B-stage to the A-stage (Matsché & Moser, 1993). [b]Capacity calculated according to 60 g BOD PE^{-1} d^{-1}. [c]136 g TOD PE^{-1} d^{-1}. [d]54 g BOD PE^{-1} d^{-1}. [e]150 g TOD PE^{-1} d^{-1}.

Country	Location	Difference with traditional AB-configuration	Design capacity (PE)	Reference
Austria	Alland petrol station	Hybrid[a]	815	(Matsché & Moser, 1993)
Austria	Egg	Hybrid	42 000	(Matsché & Winkler, 2014)
Austria	Erpfendorf	n.a.	n.a.	(de Graaff & Roest, 2012)
Austria	Flirsch	Hybrid	38 500	(Matsché & Winkler, 2014)
Austria	Hard-Hofsteig	Hybrid	138 000	(Matsché & Winkler, 2014)
Austria	Hohenems	Hybrid	170 000	(Matsché & Winkler, 2014)
Austria	Kirchbichl	Hybrid	100 000	(Matsché & Winkler, 2014)
Austria	Klosterneuburg	Hybrid	55 000	(Matsché & Winkler, 2014)
Austria	Knittelfeld	Hybrid	70 000	(Matsché & Winkler, 2014)
Austria	Radfeld	n.a.	n.a.	(Constantine et al., 2012)
Austria	Salzburg	n.a.	680 000[b]	(de Graaff & Roest, 2012)
Austria	St.Michael im Lungau	Hybrid	25 000	(Matsché & Winkler, 2014)
Austria	Strass	n.a.	200 000[c]	(Wett et al., 2007)
Austria	Vienna Main WWTP	Stand-alone HRAS with pilot Hybrid system	4 000 000[b]	(Wandl et al., 2002)
Austria	Wagram West	Hybrid	16 000	(Matsché & Winkler, 2014)
China, Guangdong	Guangzhou (Liede WWTP)	Option to run A-stage and B-stage in parallel	n.a.	(Wenyi et al., 2006)
China, Guangdong	Shenzhen (Luofang WWTP)	n.a.	650 000	(Wenyi et al., 2006)
China, Shandong	Tai'an	n.a.	n.a.	(Wenyi et al., 2006)
China, Shandong	Zibo WWTP	n.a.	n.a.	(Wenyi et al., 2006)
China, Yunnan	n.a.	n.a.	n.a.	(Wenyi et al., 2006)
Denmark	Odense	n.a.	300 000[d]	(Salomé, 1990)
Germany	Bad Schwalbach	n.a.	24 000[d]	(Salomé, 1990)
Germany	Baesweiler	n.a.	50 000[d]	(Salomé, 1990)
Germany	Bergisch-Gladbach	n.a.	n.a.	(Constantine et al., 2012)
Germany	Bettendorf	n.a.	45 000[d]	(Salomé, 1990)
Germany	Buchloe	n.a.	n.a.	(Constantine et al., 2012)
Germany	Donrath	n.a.	5 000[d]	(Salomé, 1990)

Germany	Duisdorf Bonn	n.a.	36 000[b]	(de Graaff & Roest, 2012)
Germany	Düsseldorf-Süd	n.a.	1 000 000[d]	(Salomé, 1990)
Germany	Eschweiler/Aachen	n.a.	160 000	(Haider et al., 2000)
Germany	Grefrath	n.a.	92 000[d]	(Salomé, 1990)
Germany	Hückelhoven	n.a.	50 000[d]	(Salomé, 1990)
Germany	Köln-Langel	n.a.	130 000[d]	(Salomé, 1990)
Germany	Köln-Stammheim	n.a.	1 570 000[d]	(Salomé, 1990)
Germany	Krefeld	n.a.	1 200 000	(Böhnke et al., 1997b)
Germany	Langerwehe	n.a.	15 000[d]	(Salomé, 1990)
Germany	Marktheidenfeld	n.a.	30 000[d]	(Salomé, 1990)
Germany	Neuenkirchen	n.a.	45 000[d]	(Salomé, 1990)
Germany	Neuss-Ost	n.a.	100 000[d]	(Salomé, 1990)
Germany	Neuss-Süd	n.a.	200 000[d]	(Salomé, 1990)
Germany	Rheinberg	n.a.	50 000[d]	(Salomé, 1990)
Germany	Rheinhausen	n.a.	170 000[d]	(Salomé, 1990)
Germany	Rumeln	n.a.	n.a.	(Böhnke, 1985)
Germany	Sennestadt	n.a.	25 000[d]	(Salomé, 1990)
Germany	Solingen-Ohligs	n.a.	100 000[d]	(Salomé, 1990)
Germany	Stolberg	n.a.	n.a.	(Constantine et al., 2012)
Germany	Tönisberg	n.a.	12 000[d]	(Salomé, 1990)
Korea	n.a.	n.a.	n.a.	(Schulze-Rettmer et al., 1992)
Spain	Badiolegi	n.a.	50 000	(de Graaff & Roest, 2012)
Sweden	Sjölunda	Primary settling + A-stage + trickling filter & MBBR	300 000	(Polizzi, 2013)
The Netherlands	Garmerwolde, Groningen	n.a.	375 000[e]	(de Graaff & Roest, 2012)
The Netherlands	Nieuwveer, Breda	n.a.	440 000[e]	(de Graaff & Roest, 2012)
The Netherlands	Rotterdam Dokhaven	n.a.	564 000[e]	(de Graaff & Roest, 2012)
The Netherlands	Utrecht	n.a.	480 000[e]	(de Graaff & Roest, 2012)
The Netherlands	Velsen	n.a.	136 000[c]	(de Graaff & Roest, 2012)
USA, New York	Newtown Creek WWTP, New York	Stand-alone HRAS	n.a.	(Adamski et al., 2000)
USA, Virginia	Chesapeake-Elizabeth WWTP, Virginia Beach	Stand-alone HRAS	n.a.	(DeArmond et al., 2015)
USA, Washington DC	Blue Plains Advanced WWTP	CEPT + A-stage + B-stage	6 000 000	(DC Water, 2016)

Few literature sources exist that describe operation, performance and energy demand of AB-systems. A study by de Graaff and Roest (2012) described the performance of four AB-installations from The Netherlands. **Table 1.2** summarizes some of the operational data from the A-stages of these plants.

Table 1.2: Operational details of the A-stages of four AB-installations in The Netherlands. Average data from 2010. $SRT_{A\text{-}stage}$: SRT of the contact tank, not considering the volume of the settlers. Data from de Graaff and Roest (2012).

	Contact time (min)	**$SRT_{A\text{-}stage}$ (d)**	**SRT_{aer} (d)**	**SLR (g BOD g^{-1} TSS d^{-1})**	**Reciculation factor (Q_r / Q_{in})**	**Denitrification location**
Groningen	33	0.33	0.23	2.2	0.53	A-stage & anoxic zones B-stage
Breda	23	0.65	0.19	2.7	1.6	A-stage & anoxic zones B-stage
Rotterdam	51	0.27	0.22	3.5	0.58	A-stage
Utrecht	33	0.30	0.21	2.8	0.33	A-stage & B-stage with methanol addition

	Chemical addition	**Removal percentages (%)**					**COD recovery (%)**	**Aeration energy (kWh kg^{-1} $COD_{removed}$)**
		COD	**BOD**	**Kjeldahl N**	**Total P**	**TSS**		
Groningen	$FeCl_3$	55	43	25	51	67	50	n.a.
Breda	$FeSO_4$	53	61	29	44	59	26	0.104
Rotterdam	$FeCl_3$	74	82	38	68	68	52	0.169
Utrecht	$FeClSO_4$	60	58	18	65	n.a.	41	0.090

With respect to improving the energy balance of wastewater treatment, the high-rate activated sludge process should be further optimized. COD removal in HRAS systems primarily occurs through bioflocculation and, to a lesser extent, oxidation. This makes HRAS a promising technology to explore for the purpose of energy recovery. However, adsorption and storage of soluble substrate do not always play an important role in HRAS systems with a conventional configuration ('conventional HRAS' or high-rate conventional activated sludge; HiCAS). Only 2-10% of incoming soluble COD is stored as PHB (Haider, 2002; Haider et al., 2000), and the net removal of soluble COD may be as low as 7-30% in cases where HRT, SRT and DO are not optimal (Jimenez et al., 2015; Wett et al., 2014). Some of the AB-installations still in operation today periodically struggle with bioflocculation issues in the A-stage (WBD, 2012, personal communication with plant operators). The washout of particles from the A-stage results in a COD recovery and a higher aeration requirement in the B-stage. This may make it challenging for HRAS systems to compete with less complex, non-biological processes for energy recovery from wastewater, such as primary settling (Wett et al., 2014). The process of chemically enhanced primary treatment (CEPT), where chemical coagulation and flocculation are applied to improve the recovery of organic matter, has a good potential to energy and nutrient recovery from wastewater (Diamantis et al., 2013). Primary settling and CEPT can achieve a particulate matter removal of up to 70 and 90%, respectively (Metcalf & Eddy, 2003). However, the processes are not optimized for removal of dissolved organics, which limits the maximum amount that can be recovered if the wastewater contains a large fraction of dissolved COD. Additionally, CEPT

may suffer from high operational expenses and reduced digestibility of the sludge, similar to the case of chemical phosphorus precipitation (see section 3.3).

5.4 High-rate contact stabilization

In order to make HRAS an efficient, reliable and economically competitive technology for energy recovery from wastewater, the adsorption and storage capacity of the system needs to be enhanced. Efforts have been made to maximize sludge production by exploring process configurations as diverse as (1) conventional HRAS (HiCAS) systems, in which operational parameters such as oxygen concentrations and retention times are optimized (Jimenez et al., 2015; Rahman et al., 2015; Wett et al., 2014), (2) high-rate membrane bioreactors (MBRs), in which efficient solid/liquid separation is imposed by means of membrane filtration (Akanyeti et al., 2010; Faust et al., 2014b; Hernandez Leal et al., 2010), and (3) dissolved air flotation (DAF), in which solid/liquid separation is achieved by stimulating air bubble formation on the suspended particles (Ding et al., 2015a). Technologies to improve the solid/liquid separation beyond what can be achieved by gravitational settling, such as MBR and DAF technology, bear the disadvantage of additional operational costs. Furthermore, none of the above processes subjects the sludge microorganisms to a selection pressure to improve biological adsorption and storage.

In the search for a HRAS process capable of recovering particulate, colloidal as well as dissolved COD as sludge, it is proposed that HRAS technology be combined with a feast-famine regime to stimulate substrate biosorption. One of the few examples that attempts to combine a HRAS system with a (modest) feast-famine regime is the Hybrid© system (Matsché & Moser, 1993). This is an AB-system with partial exchange of A-sludge to the B-stage and vice versa, and therefore subjects a minor part of the sludge to feast-famine (Wandl et al., 2002). However, the Hybrid system is not well characterized and it remains unknown as to what extent its modest feast-famine regime results in an improved sorption and storage capacity. In this work, I propose to completely subject a high-rate system to a feast-famine regime in order to improve its sorption and storage capacity. Such a system would have a similar configuration as the contact stabilization system (see section 5.1.2) and is therefore named the high-rate contact stabilization (HiCS) process. Exploratory studies have been performed in the past on HiCS-like configurations (Huang & Li, 2000; Zhao et al., 2000). These systems showed a promising removal of COD, and it was indicated that, given optimization, the HiCS could compete with HiCAS systems in terms of COD removal. Moreover, similar to the low-rate contact-stabilization process, the relatively high sludge concentrations during stabilization may result in lower overall reactor volume requirements and lower investment costs compared to conventional systems.

No additional studies have been performed on high-rate contact stabilization. It has not been studied how much of the overall COD removal in the HiCS system is attributable to oxidation, and whether the overall COD balance of the HiCS system is favorable enough to outperform HiCAS systems in terms of energy recovery. It remains to be investigated how much the sludge adsorption and/or storage capacity can be improved in a HiCS system compared to a HiCAS system.

6 Objectives

6.1 Recapitulation

The anthropogenic water cycle is unsustainable in its current form (see sections 1 and 2).

Conventional wastewater treatment is energetically inefficient and is designed to remove resources such as organic matter and nutrients, without recovering them (see section 3).

The microbial ecology of activated sludge has primarily been studied on a descriptive basis. Little is known about the causal influence of operational parameters on the microbial community and its functional outputs. This is especially the case for high-rate systems (see section 4).

In the quest for sustainable wastewater treatment with resource recovery, high-rate activated sludge plays a major role. For a more efficient and viable energy recovery from wastewater, the adsorption and storage capacity of HRAS sludge needs to be improved (see section 5).

High-rate contact stabilization (HiCS) can be a promising technology to achieve improved adsorption and storage in a high-rate process, but the HiCS system has not been explored in this context (see section 5.4).

6.2 Outline of this thesis

This thesis aims to respond to the issues presented above. This work is constructed along four research chapters and a general discussion (**Figure 1.7**).

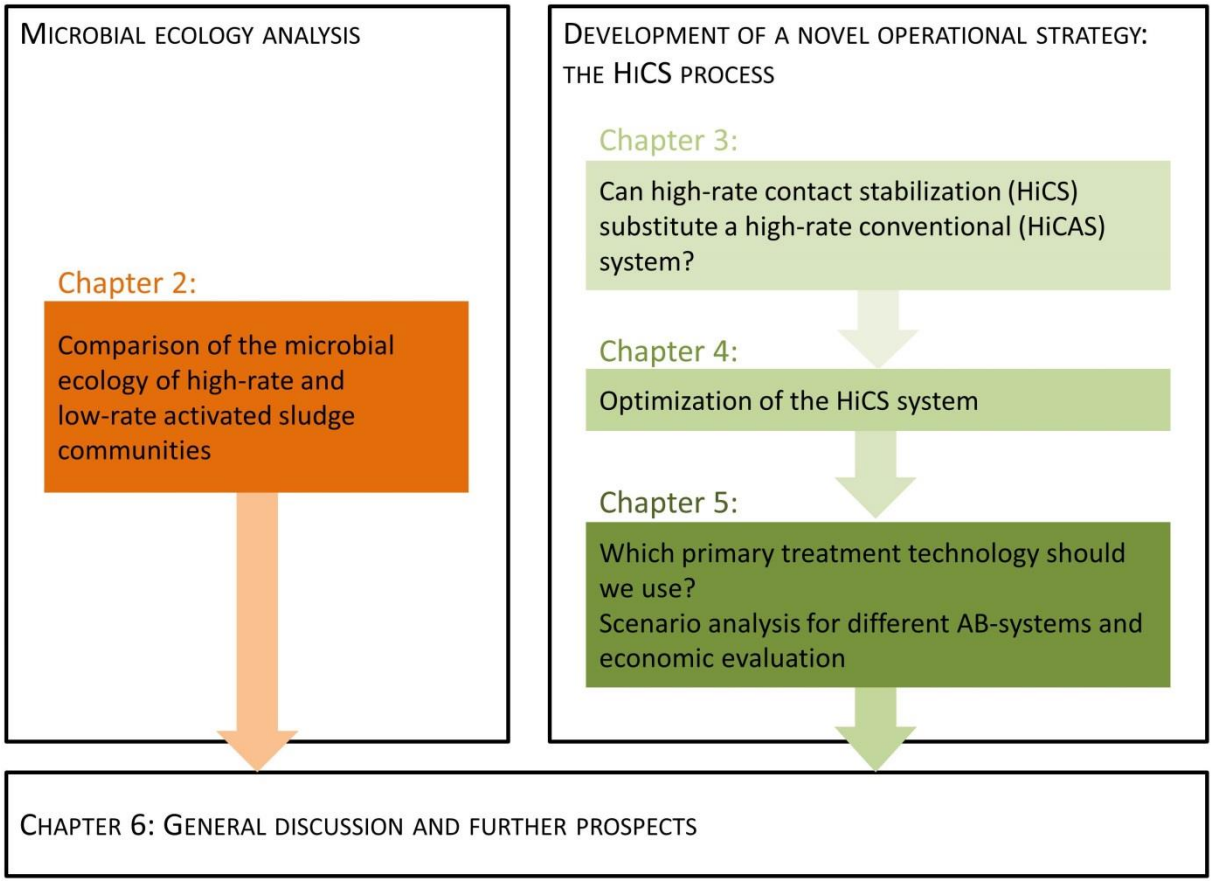


Figure 1.7: Outline of the next chapters

- **Chapter 2** presents an exploration of the microbial ecology of high-rate activated sludge communities on a fundamental level. The structure and dynamics of a high-rate community were compared to those of a low-rate community in a year-round follow-up of a full-scale AB-installation. The aim of this study was to identify key differences between high-rate and low-rate communities that may have implications on the way these systems can be engineered and controlled.
- **Chapter 3** compares the performance of a HiCS system with a conventional HRAS (HiCAS) system on a laboratory scale, both with synthetic as well as real wastewater. The aim of this study, in which the HiCS system was not yet optimized, was to provide a preliminary comparison between both systems and investigate whether HiCS could potentially replace HiCAS as a preferred technology for energy recovery.
- **Chapter 4** presents the results of optimization experiments on the HiCS system treating high-strength wastewater. Different reactor experiments were performed on a laboratory scale with synthetic wastewater. Three operational parameters were varied: SRT, HRT in the contact phase and HRT in the stabilization phase. The aim of this study was to select the operational strategy that yielded the highest recovery of organic matter as sludge.

- **Chapter 5** presents experiments to determine the performance changes when switching between a HiCAS and HiCS configuration in a high-rate system treating medium-strength wastewater. Subsequently, a detailed economic analysis is provided for upgrading a stand-alone CAS installation to an AB-system. Different technologies were considered for the primary stage (primary settling, conventional A-stage and HiCS), intermediate settling (addition or no addition of coagulants) and the B-stage (N/DN, nit/denit, PN/A). The aim of this study was to evaluate which combination of technologies would result in an optimal wastewater treatment, in terms of energy balance, investment costs and operational costs.
- **Chapter 6** presents a general discussion and outlooks for further research. First, the state of progress is summarized, followed by a discussion on microbial resource management of high-rate systems, prospects for further technological optimization of the HiCS system, and mathematical modeling of the HiCS process. The chapter finishes with an outlook of the merits of high-rate activated sludge beyond its application for energy recovery, and a general conclusion.

CHAPTER 2:

COMPARISON OF THE MICROBIAL ECOLOGY OF HIGH-RATE AND LOW-RATE ACTIVATED SLUDGE COMMUNITIES

Cover figure: Strange constellations in the night sky above the Breda STP.

This chapter has been redrafted after:

MEERBURG, F. A., VLAEMINCK, S. E., ROUME, H., SEUNTJENS, D., PIEPER, D. H., JAUREGUI, R., VILCHEZ-VARGAS, R. & BOON, N. (2016). High-rate activated sludge communities have a distinctly different structure compared to low-rate sludge communities, and are less sensitive towards environmental and operational variables. *Water Research* **100**: 137-145.

1 Introduction

Activated sludge treatment plays a central role in the management of domestic wastewater (sewage) and industrial wastewaters. While the conventional activated sludge process has proven its merits in terms of reliability and performance, it suffers from drawbacks such as high operational costs and limited potential for resource recovery. In recent years, high-rate activated sludge (HRAS) processes have gained attention because of their potential use for recovery of energy and organics from sewage, both as stand-alone processes or as part of an AB-system (see Chapter 1). The HRAS process shows a great potential to improve the net energy balance of sewage treatment. In temperate and colder climates, high-rate activated sludge treatment may be the most economically viable technology to achieve up-concentration of organics from sewage for subsequent recovery (Verstraete et al., 2009; Verstraete & Vlaeminck, 2011).

Despite ever-improving process control, many sewage treatment plants (STPs) still struggle with operational problems that may coincide with changes in the microbial community (Briones & Raskin, 2003; Gentile et al., 2007). Both for high-rate as well as conventional, low-rate systems, there is a need for better knowledge of the activated sludge community in relation to its dynamics, functional output and sensitivity toward external factors, such as changes in environmental conditions. With the development of advanced molecular techniques, a number of studies has monitored the community structure and dynamics of activated sludge over relatively long time periods, and explored interactions of microbial species with environmental factors, with other microbial species, and with the functional output of the system (Ju & Zhang, 2014; Ofiţeru et al., 2010; Valentin-Vargas et al., 2012). Much remains unknown, however, about the differences between high-rate and low-rate communities in all of these aspects.

In microbial ecology, the traditional niche theory holds that microbial communities are shaped by deterministic – i.e., predictable – factors, such as environmental conditions (Chase & Leibold, 2003). Changes in, for example, temperature, can have a determined influence on a species' growth rate. Different species may have different 'niches' or combinations of environmental conditions that are optimal for their growth. Thus, according to the niche theory, changes in environmental conditions will cause a shift in microbial community structure in a deterministic manner. This niche theory has been challenged by the concept of neutral change, which is based on the theory of island biogeography with a dynamic equilibrium between extinction and colonization (Hubbell, 2001). According to the theory of neutral community assembly, changes in microbial communities primarily reflect 'stochastic' or chance-driven processes. In other words, species may enter or disappear from a community as a result of natural fluctuations of their abundance over time, without underlying influences of environmental conditions. Recent studies suggest that activated sludge communities are shaped by both deterministic and neutral factors (Ayarza & Erijman, 2011; Ofiţeru et al., 2010; Valentin-Vargas et al., 2012). Little to no knowledge exists about how the relative importance of deterministic versus neutral dynamics differs between high-rate and low-rate communities.

Microbial communities are generally composed of a relatively small number of abundant species and a large number of rare species (Sogin et al., 2006). It is theorized that abundant species play a functional role in the ecosystem, while rare species merely act as a 'seed bank', i.e., a reserve of species present at low abundances and low activities that may become more abundant and active when conditions change (Lennon & Jones, 2011; Pedros-Alio, 2012). This may not be a general rule. For example, certain nitrifiers have been found in activated sludge at low abundance based on DNA concentrations, despite high transcription activity of nitrification-associated genes (Yu & Zhang, 2012). Previous research has found that abundant sub-communities in activated sludge are less diverse than rare sub-communities and have lower species turnover rates, as indicated by the average number of new species entering the respective sub-communities per unit of time (Kim et al., 2013). However, little is known about differences in species-species and species-environment interactions between abundant and rare sub-communities, and how these interactions might differ between high-rate and low-rate communities.

While gradual progress is made in understanding the microbial ecology of conventional activated sludge systems, a large knowledge gap exists concerning high-rate activated sludge communities and their structure, dynamics, and sensitivity towards environmental factors. In this work, the high-rate and low-rate activated sludge communities of a two-stage STP were studied, and systematically compared over a period of 10 months. This work addresses four questions concerning differences in microbial ecology between high-rate and low-rate systems: (1) Are high-rate and low-rate systems distinctly different in terms of community structure? (2) Are high-rate community dynamics more variable and less governed by deterministic factors compared to low-rate communities? (3) Are community shifts in abundant and transitional sub-communities more deterministic than shifts in rare sub-communities? And (4) do high-rate community members show a lower co-occurrence and lower correlation with environmental variables than low-rate community members?

2 Material and Methods

2.1 Plant description and sampling

The Nieuwveer STP in Breda (The Netherlands) operates an AB-process, and treats combined domestic and industrial wastewater from Breda and neighboring municipalities. The plant was designed for a capacity of 400,000 population equivalents and the average influent flow rate during the study period was 80,100 m^3 d^{-1}. The high-rate stage consists of a 3,500 m^3 basin with an anoxic, a facultative oxic and an oxic segment. The low-rate stage treats the high-rate effluent. It consists of four parallel basins, of which the first three have a volume of 5,400 m^3 and a segment train of one anoxic, two facultative oxic, two oxic and again one facultative oxic segment. The fourth basin has a volume of 12,000 m^3 and a segment train of two anoxic, four facultative oxic and four oxic segments. The high-rate and low-rate stages have a separate sludge recycle, each with a designed sludge recycle ratio ($Q_{recycled}$ $Q_{influent}^{-1}$) of 0.5. At the time of the study, final effluent was recirculated back to

the plant inlet for improved denitrification, with a measured effluent recirculation factor ($Q_{recirculated}$ $Q_{influent}^{-1}$) between 0.1 and 3.6. From October 2013 to July 2014, near-weekly sludge samples (60 mL) were taken from the sludge recycle stream of the high-rate system and from the first segment of the largest low-rate basin. Samples were immediately centrifuged (10 min at 4,000*g*). After manual homogenization of the pellets, subsamples of 0.5 mL pelletized sludge were frozen at -20°C for transport and stored at -80°C until further processing. In parallel, fresh suspended sludge samples (1 L) were transported to the lab for additional analyses within 24 h.

2.2 Environmental and operational data

Environmental and operational data were obtained from Waterschap Brabantse Delta (The Netherlands), who manage the STP. Total suspended solids (TSS), VSS, chemical oxygen demand (COD), BOD, nitrite, nitrate, Kjeldahl nitrogen (KjN) and phosphorus concentrations were determined by Waterschap Brabantse Delta according to standard methods (Greenberg et al., 1992). The sludge volume index (SVI) was measured after 30 settling in an Imhoff cone, using undiluted sludge from the plant (i.e., the TSS concentration was unaltered). Volume-weighted average diameters ($D_{4,3}$) of the sludge flocs were measured with a Mastersizer S (Malvern, Malvern, UK), as described by Courtens et al. (2014). Extracellular polymeric substances (EPS) were extracted from the sludge flocs using a heat extraction protocol described by Judd and Judd (2006) and subsequently stored at -20°C. For determination of the EPS protein content, samples were alkalified to a final concentration of 1 M NaOH, and analyzed using the Lowry protein assay (Lowry et al., 1951) with bovine serum albumin as a standard.

Data collection of environmental and operational variables did not always coincide with sampling of the microbial communities. For continuously measured variables such as temperature, recirculation factor, hydraulic residence time, oxygen concentrations and rainfall, average values were taken for a two-day interval before each sludge sample. For the intermittently measured variables, the value closest in time to each sludge sample was used within a range of a few days before to 1 day after sludge sampling. **Table 2.1** lists all environmental and operational variables used in this study, and their abbreviations.

Table 2.1: Average values of environmental and operational variables throughout the study period, with standard deviations. Averages that differ by more than a factor two between the high- and low-rate system are indicated in bold. n = number of data points. The *p*-values indicate the significance level of pairwise comparisons between the high-rate and low-rate values.

Environmental variables	**Abbreviation**	**High-rate**	**Low-rate**		**n**	***p*-value**
Day of sampling	Time	Day 0 (Oct 2013) to 273 (Jul 2014)		d	38	
Temperature	Temperature	10.1 (min) - 20.4 (max)		°C	38	
Rainfall	Rainfall	0 (min) - 13.4 (max)		mm/d	38	
Recirculation factor of final effluent back to influent	R.factor	1.3 ± 0.7		fraction	38	
BOD concentration of influent	**BOD**	**100.4 ± 29.2**	**47.7 ± 12.6**	**mg L^{-1}**	**37**	**1.58 x 10^{-13}**
Floc size (volume-weighted average diameter)	**$D_{4,3}$**	**256.7 ± 83.6**	**87.1 ± 8.3**	**µm**	**27**	**3.38 x 10^{-11}**
Hydraulic retention time (nominal)	**HRT_{nom}**	**0.024 ± 0.012**	**0.188 ± 0.102**	**d**	**38**	**5.57 x 10^{-12}**
Sludge retention time of reactor + settling system	**SRT_{syst}**	**1.74 ± 0.53**	**34.4 ± 28.8**	**d**	**37**	**4.49 x 10^{-8}**
Sludge-specific loading rate	**SLR**	**2.13 ± 0.67**	**0.11 ± 0.03**	**g BOD g^{-1} VSS d^{-1}**	**37**	**8.33 x 10^{-20}**
COD removal efficiency	COD.removed	0.54 ± 0.11	0.70 ± 0.07	fraction	37	2.22 x 10^{-10}
COD/N ratio of influent	COD/N	10.8 ± 2.1	6.3 ± 1.3	mg mg^{-1}	37	2.59 x 10^{-16}
Kjeldahl nitrogen concentration of influent	KjN	23.2 ± 4.7	20.8 ± 3.8	mg L^{-1}	37	1.58 x 10^{-2}
Nitrogen removal efficiency	N.removed	0.33 ± 0.11	0.52 ± 0.12	fraction	37	3.48 x 10^{-10}
Observed sludge growth yield	Y_{obs}	0.67 ± 0.23	0.50 ± 0.54	g TSS g^{-1} COD	37	n.s.
Phosphorus concentration (incoming)	P	4.3 ± 1.1	3.0 ± 1.2	mg L^{-1}	37	3.14 x 10^{-6}
Phosphorus removal efficiency	P.removed	0.47 ± 0.18	0.45 ± 0.16	fraction	37	n.s.
Proteinaceous extracellular polymeric substances	EPS.P	37.6 ± 8.2	73.9 ± 26.3	mg BSA g^{-1} VSS	17	3.49 x 10^{-5}
Sludge volume index	SVI	76.5 ± 14.3	120.1 ± 15.9	mL g^{-1}	38	4.06 x 10^{-20}
TSS concentration	TSS	2780 ± 545	3371 ± 444	mg L^{-1}	38	1.62 x 10^{-6}
VSS/TSS ratio in high-rate system	VSS.TSS	0.79 ± 0.04	n.a.	fraction	38	
Oxygen concentration in second compartment high-rate	O_2.A2	0.44 ± 0.21	n.a.	mg L^{-1}	38	
Oxygen concentration in third compartment high-rate	O_2.A3	1.74 ± 0.55	n.a.	mg L^{-1}	38	
Recirculated nitrate to high-rate system	N.recirculated	3.60 ± 1.52	n.a.	mg L^{-1}	38	

2.3 Community analysis

DNA was extracted from the pelletized sludge samples using a FastPrep-24 system (MP Biomedicals, California, USA), and precipitated according to the protocol described by Vilchez-Vargas et al. (2013). The DNA pellets were resuspended in 100 µL MilliQ water. The quantity of the DNA was tested by monitoring the absorbance at 260 nm and absorbance ratios at 260 nm and 280 nm using a NanoDrop ND-1000 (Thermo Scientific, Massachusetts, USA), and the quality was checked by electrophoresis on a 1% (w/v) agarose gel. Samples were sequenced using the high-throughput MiSeq Illumina platform (Illumina, California, USA). Regions V5-V6 of the 16S rRNA gene were amplified, and targeted with adapters and barcodes suitable for Illumina sequencing, as previously described (Bohorquez et al., 2012; Camarinha-Silva et al., 2014). Quality filtering was performed as described by Camarinha-Silva et al. (2014). Read length was between 140 and 273 nucleotides. Reads were clustered using the Mothur pipeline (Schloss et al., 2009), allowing two mismatches. This resulted in 1,677 unique taxa (phylotypes). The phyloseq package (McMurdie & Holmes, 2013) was used in R (version 3.0.2) to randomly normalize each sample to the minimum sequencing depth of 15,186 reads, and the vegan package (Oksanen et al., 2013) was used to visualize that all samples reached a plateau in the rarefaction curve (**Figure 2.1**). Phylotypes were annotated in the RDP classifier (Cole et al., 2014) using the naïve Bayesian classification (Wang et al., 2007) with a threshold of 80%, and manually analyzed using the seqmatch function. A taxonomic level was only assigned when 16S rRNA gene fragments of previously described isolates or uncultured representatives of that taxon showed ≤ 2 mismatches. Sequences were deposited in the European Nucleotide Archive (accession numbers LT217663-LT219428).

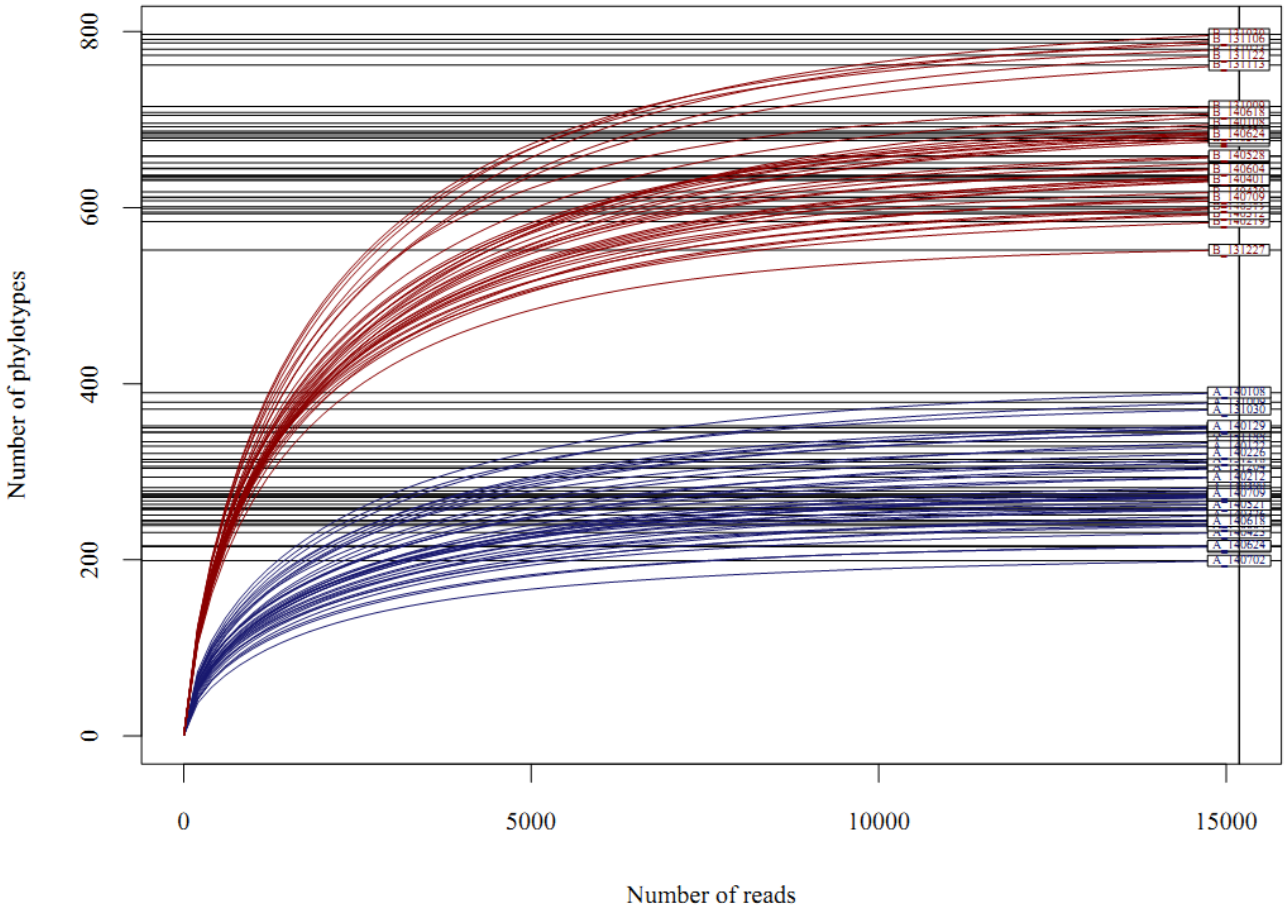


Figure 2.1: Rarefaction curve displaying the number of phylotypes against the depth of each of the high-rate (blue) and low-rate (red) samples.

2.4 Statistical analysis

Statistical comparisons of community indices (richness, evenness, dynamics and relative phylum abundance) between the high-rate and low-rate systems were performed in R. The Shapiro-Wilk test was used to test the normality of the data residuals. The null hypothesis of normality was rejected for the evenness and dynamics of the high-rate system, and for some of the relative phylum abundances in the high-rate and low-rate systems. Therefore, pairwise statistical comparisons of community indices between the high-rate and low-rate systems were performed using the Mann-Whitney *U* test as a non-parametric alternative for the Student's *t*-test. Differences were considered significant at a *p*-value below 0.05. Ordination and calculation of diversity and dissimilarity indices were performed using the vegan package in R. Unimodal ordination methods (correspondence analysis, CA; and canonical correspondence analysis, CCA) were preferred, since the gradient lengths of the detrended correspondence analyses were always ≈ 4 (Ramette, 2007). For all ordinations, only environmental variables that significantly correlated to the unconstrained CA axes (9999 permutations) were considered for variation partitioning in CCA analysis. Pearson and Spearman correlations were calculated using the hmisc package in R (Harrell, 2014). To construct co-occurrence

networks, the absolute phylotype (Phy) abundance matrices were used to calculate Pearson correlations in a pair-wise manner. Only significant correlations above 0.65 were used for network construction. The undirected network was visualized and analyzed using Cytoscape (version 3.2.1) (Shannon et al., 2003), using an organic layout.

3 Results and Discussion

3.1 Question 1

"Are high-rate and low-rate systems distinctly different in terms of community structure?"

A total of 22 environmental and process variables were monitored for the high-rate and 19 for the low-rate systems of the sewage treatment plant (**Table 2.1**). The main differences between the two systems were the incoming BOD concentration, the SLR and the $D_{4,3}$, which were considerably higher in the high-rate system, and the HRT and SRT, which were considerably shorter. The high-rate system achieved an average COD removal of 54%, as opposed to 70% in the low-rate system. This may be a result of the shorter SRT of the high-rate system, which limits the fraction of substrate that can be degraded by high-rate sludge compared to low-rate sludge (Haider et al., 2003). Throughout the study period, no major disruptions of plant performance occurred, and the STP was able to remove 85-96% of COD and 95-99% of TSS. Removal performances of nitrogen (42-91%) and phosphorus (33-95%) were more variable, with minima occurring between the colder months of November 2013 to February 2014.

CA ordination of the phylotype-sample abundance matrices showed a clear separation between samples of the high-rate and low-rate systems along the primary ordination axis, while the secondary axis showed variation within each stage. A major fraction of the phylotypes also clustered according to a similar pattern (**Figure 2.2**). Fitted environmental variables indicate the direction of each variable across the ordination space, and their length reflects the strength of correlation to the ordination axes. The distinction between samples and phylotypes along the first ordination axis was most strongly correlated to the environmental variables of HRT_{nom}, SRT_{syst}, SLR, COD/N ratio, $D_{4,3}$, SVI, BOD and TSS. Variation along the second ordination axis was most strongly correlated to the time.

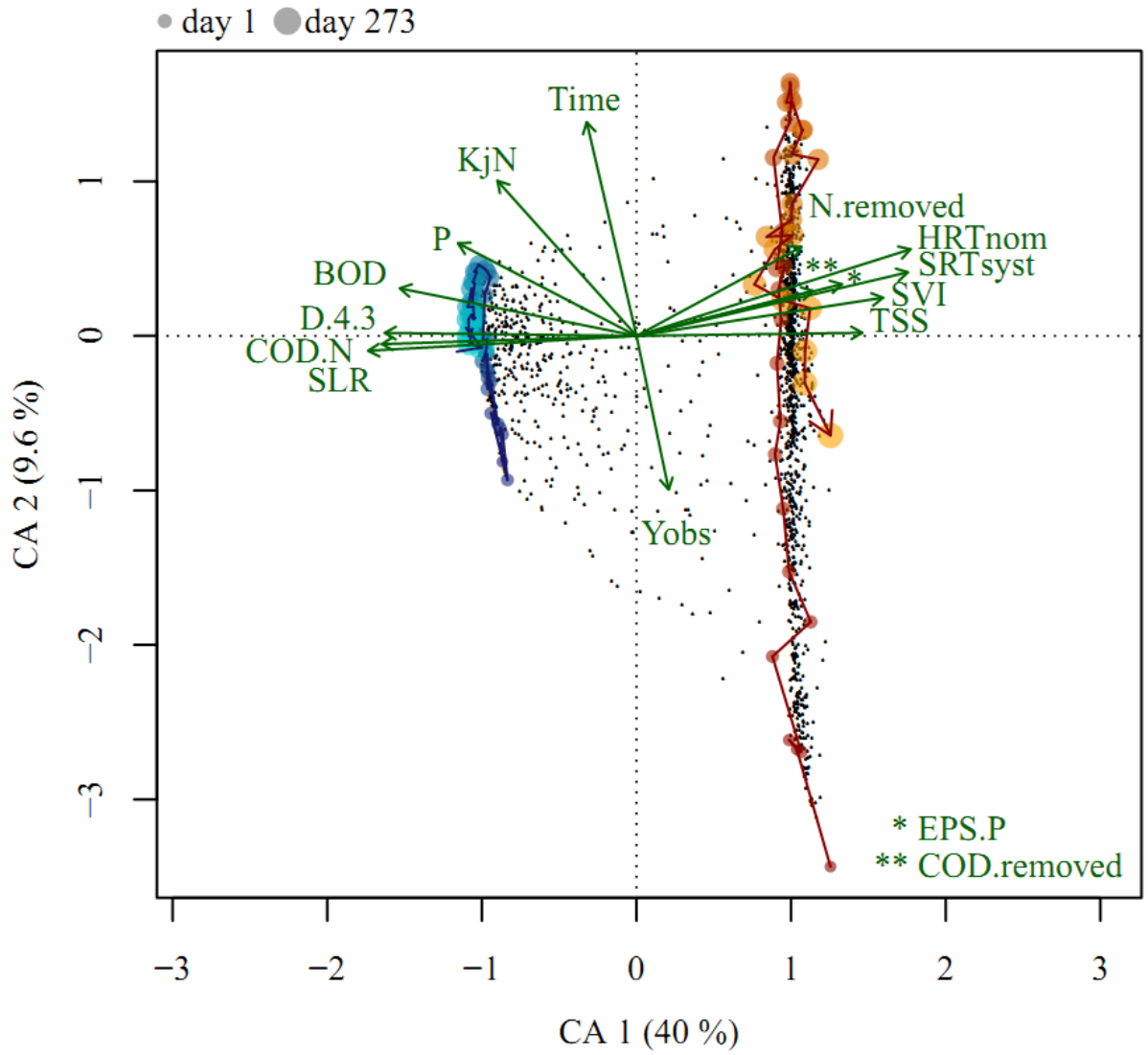


Figure 2.2: Correspondence analysis (CA) of the combined high-rate (blue) and low-rate (red) communities from October 2013 to July 2014. Phylotypes are shown as dots. Samples are shown as circles with increasing size in chronological order, and connected by a blue or red arrow. Environmental variables that significantly correlate to the ordination are plotted as green arrows. Abbreviations are the same as in **Table 2.1**. Percentages indicate the relative contribution of each axis to total inertia.

Over the entire sampling period, 266 phylotypes were detected uniquely in the high-rate system, 990 uniquely in the low-rate system, and 510 phylotypes were detected at least in one sample of both stages. Community-wide comparison showed that the high-rate system had a considerably lower observed richness (289 ± 48 phylotypes) and Pielou's evenness (0.62 ± 0.06), compared to the low-rate system (668 ± 63 phylotypes and 0.82 ± 0.02, respectively) (**Figure 2.3**), and these differences were highly significant ($p < 10^{-12}$). Note that, for the high-rate system, species richness and evenness gradually decreased over time (correlation coefficient r = -0.77 and -0.72, respectively). Apart from

this, the richness and evenness of the high-rate and low-rate communities were not correlated to any of the environmental and operational variables included in this study.

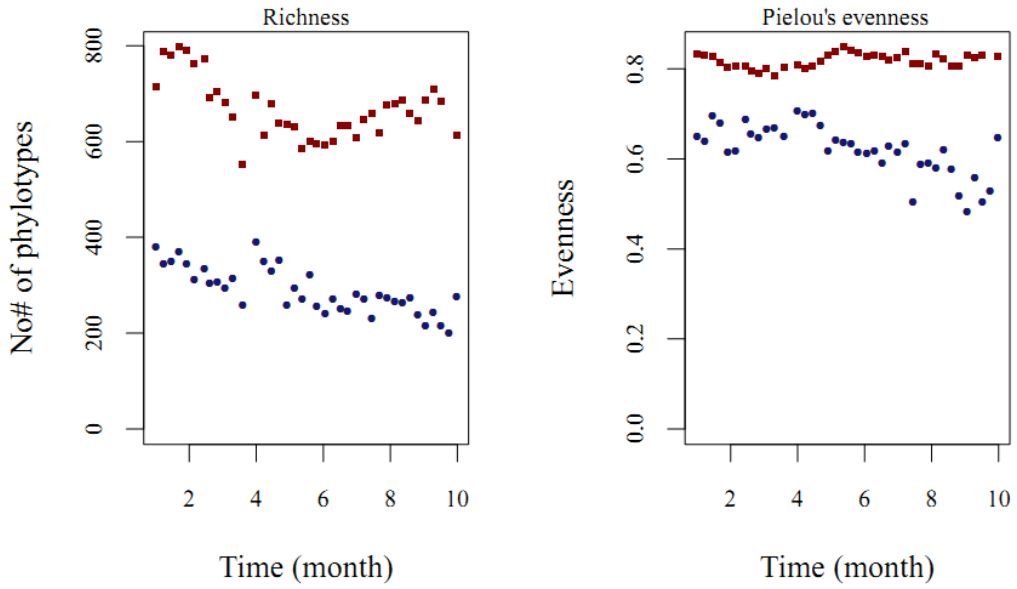


Figure 2.3: Richness and Pielou's evenness for each sample of the high-rate (•) and low-rate (▪) system.

These results are complementary to a recent study of ten single-time-point samples from different high-rate and low-rate STPs (Gonzalez-Martinez et al., 2016), which showed that, of the five studied environmental variables, the SRT and HRT were most strongly correlated with differences in microbial community structure. However, mentioned study did not incorporate several environmental factors that were shown in current study to associate with differences in microbial community structure between high-rate and low-rate activated sludge (see above), including time. Gonzalez-Martinez et al. (2016) also demonstrated that the microbial communities of the high-rate sludge plants were consistently less diverse than the low-rate communities. Saikaly and Oerther (2004), argued that species richness increases with SRT. However, experimental studies on membrane bioreactors (MBRs) have demonstrated positive (Duan et al., 2009), negative (Saikaly et al., 2005) and neutral effects (Bagchi et al., 2015; Tan et al., 2008; Teksoy Başaran et al., 2014) of SRTs between 0.5 and 33 d on community richness and evenness. Besides the SRT, the evenness in the low-rate reactor may also explain its higher species richness, since systems with higher evenness are theorized to provide more niche space for microbial colonization (van der Gast et al., 2006). A study on two full-scale sewage treatment plants with large differences in SRT and SLR showed that samples from the two reactors clustered separately in CCA, and that differences in community composition could be correlated to the SRT, SLR, HRT and temperature (Valentin-Vargas et al., 2012). Neutral factors are also known to influence activated sludge communities (Valentin-Vargas et al.,

2012; van der Gast et al., 2008). Nonetheless, random factors alone cannot explain the differences in the sludge communities described in this study, considering that the hydraulic connection of the two systems creates a continuous cross-inoculation, and that differences in community structure are pronounced and consistent over time. This raises the question as to how community structure and function of high-rate and low-rate systems are affected when a substantial amount of biomass is continuously transferred from one system to another, as is the case in the Hybrid® process (Winkler et al., 2004). To exploit the full capacity of a two-stage STP, one may argue that it is essential that both stages have distinctly different microbial communities to be better adapted to the specific purpose of each stage. In this study, it was clear that the community structure and composition of the high-rate and low-rate systems were distinctly and consistently different, and that this could be attributed to differences in operational and environmental factors.

3.2 Question 2

"Are high-rate community dynamics more variable and less governed by deterministic factors compared to low-rate communities?"

The observed community dynamics were expressed as dissimilarity between consecutive samples in a moving-window approach with a fixed one-week interval (**Figure 2.4**). The high-rate system experienced an alternation between periods of stronger changes and more stable periods, whereas the low-rate system displayed a more consistent level of dynamics over time. Remarkably, the average dynamics in the two systems was similar, with a weekly Bray-Curtis dissimilarity of 0.19 ± 0.06 in the high-rate system and 0.20 ± 0.03 in the low-rate system ($p > 0.05$). At short SRT, and thus high specific growth rate, it has been suggested that sludge systems experience a higher degree of dynamics, due to oscillations in population abundances (Curtis et al., 2003; Saikaly & Oerther, 2004) and a number of studies has found a correlation between short SRT and higher community dynamics (Duan et al., 2009; Valentin-Vargas et al., 2012). On the other hand, systems with a higher diversity are thought to harbor more redundancy within functional groups (Briones & Raskin, 2003), and richer systems may therefore experience dynamic population changes without affecting functional stability. Possibly, the similar degree of dynamics for the high-rate and low-rate systems in this study was a result of the conflicting effects of SRT and diversity on system dynamics. None of the environmental and operational variables included in this study showed a significant correlation with community dynamics.

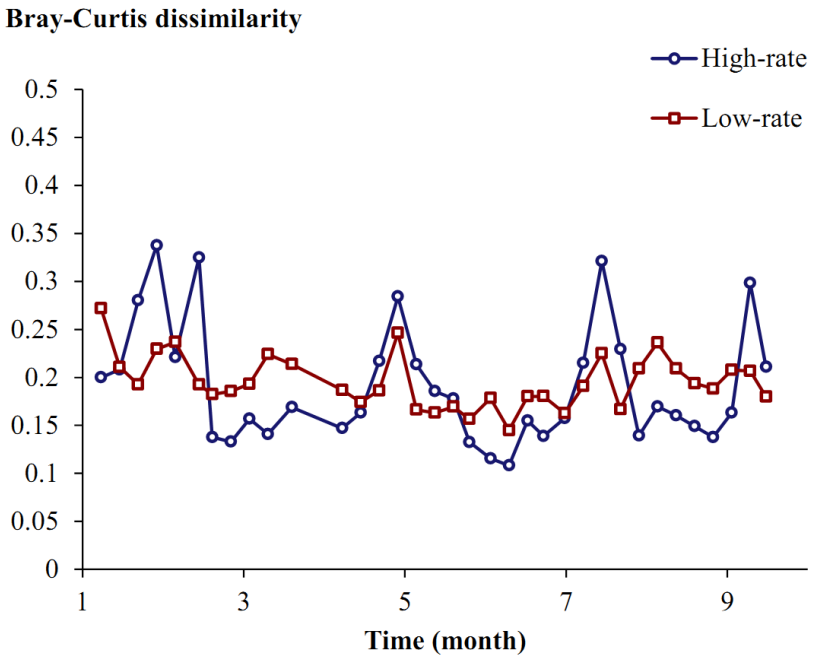


Figure 2.4: Moving-window analysis of the Bray-Curtis dissimilarity between samples with a one-week interval, for the high-rate (blue) and low-rate (red) communities.

The taxa-time relationship describes the accumulation of new phylotypes over time, and may be described by a power-law function:

$$S = ct^w \qquad \text{(Equation 2.1)}$$

where *S* is the cumulative number of taxa over time t, *c* is a constant denoting the sample richness at time t=0, and *w* is the temporal scaling exponent (Preston, 1960), which is a measure of relative species turnover rate. The temporal scaling exponents for the high-rate (0.262±0.058, R^2 = 0.960) and low-rate system (0.249±0.008, R^2 = 0.968) were similar (p > 0.05) (**Figure 2.5**), and fell within the lower range of values between 0.21 and 0.50 reported for activated sludge systems (Hai et al., 2014; Ibarbalz et al., 2014; Kim et al., 2013; Shade et al., 2013; Wells et al., 2011).

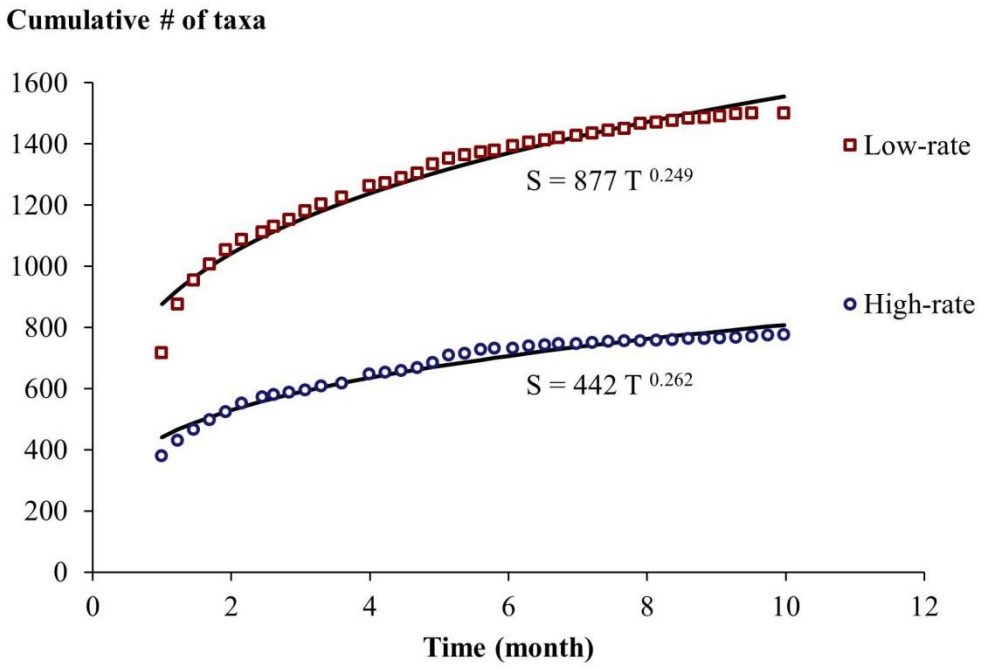


Figure 2.5: Taxa-time relationship for the high-rate (blue) and low-rate (red) system. The curves show the least-squares nonlinear regression fit of the power law of Equation 2.1.

The similarity of temporal scaling exponents of the high- and low-rate community is unexpected, given that these systems differed in species richness and selective pressure caused by differences in SRT. For example, Ayarza and Erijman (2011) found that activated sludge communities with a more diverse initial richness experienced higher species turnover rates. In contrast, van der Gast et al. (2008) reported lower turnover rates as activated sludge communities experienced a higher selective pressure. In this work, the high-rate system had a lower species richness, which would be expected to lead to lower turnover rates. Additionally, the high-rate system had a higher selective pressure on microbial growth rates because of the shorter SRT, which would also be expected to lead to lower turnover rates. The fact that community dynamics and relative species turnover rate were very similar in the high-rate and low-rate systems may therefore indicate that other factors exist, besides species richness and SRT, that influence community turnover rates, such as the higher COD availability in the high-rate system, which might dampen substrate competition between microorganisms, causing metabolic redundancy and this higher turnover rates in the high-rate system. On the other hand the seeding of microorganisms from the high-rate to the low-rate system, which may boost turnover rates in the low-rate system.

To quantify the relative importance of deterministic factors shaping the overall community structure, variation partitioning was performed by CCA ordination of the high-rate and low-rate communities separately (**Table 2.2**). Note that time may not be a true environmental factor, and community changes over time may reflect deterministic as well as neutral changes (Lynch & Neufeld, 2015).

Table 2.2: Variation partitioning using canonical correspondence analysis (CCA) on the total community of the high-rate and low-rate system, and of the three sub-communities. For each CCA analysis, only those environmental variables were included that correlated significantly to the ordination axes of an unconstrained correspondence analysis.

	High-rate system		Low-rate system	
	Significant variables	**% of variation**	**Significant variables**	**% of variation**
Total	Time, HRT_{nom}, KjN, P, Temperature, Y_{obs}	47.5%	BOD, $D_{4,3}$, Time, HRT_{nom}, KjN, SVI, Temperature	55.9%
Continuously abundant	Time, HRT_{nom}, KjN, P, Temperature	45.1%	BOD, $D_{4,3}$, Time, HRT_{nom}, KjN, N.removed, Temperature	60.6%
Transitional	Time, HRT_{nom}, KjN, P, Temperature, Y_{obs}	51.0%	BOD, $D_{4,3}$, Time, HRT_{nom}, KjN, SVI, Temperature	60.1%
Continuously rare	Time, HRT_{nom}, KjN, Temperature, Y_{obs}	28.5%	BOD, $D_{4,3}$, Time, HRT_{nom}, KjN, SVI, Temperature	44.4%

High-rate systems are generally considered incapable of extensive nitrification, due to the short SRT (Böhnke et al., 1997c; Henze et al., 2008). In this regard, it is remarkable that the influent KjN concentration explains a significant portion of each of the high-rate sub-communities, which may also be illustrated by the strongly negative correlation between the KjN concentration and *Rhodoferax*, a keystone species in the high-rate community capable of denitrification (see Section 3.4). Possibly, the influence of influent nitrogen on the high-rate community is a result of the recirculation of final effluent from the STP to the high-rate system, which is performed to allow denitrification in the high-rate system and, thus, ensure a sufficient degree of overall nitrogen removal in the plant. It should be noted, however, that both the recirculation factor of final effluent as well as the incoming NO_3^--N concentration in the A-stage never correlated strongly to any other environmental variable, any phylotype, and did not significantly explain the overall community variation in the high-rate system. Therefore, the impact of effluent recirculation on the high-rate community is likely limited.

Assuming that this study included the environmental variables most relevant for the ecology of activated sludge communities (Hai et al., 2014; Ibarbalz et al., 2014; Valentin-Vargas et al., 2012; Wells et al., 2011), the percentage of unexplained variation was 52.5% in the high-rate and 44.1% in the low-rate system. This suggests that high-rate activated sludge communities are more shaped by neutral factors than low-rate communities. Possibly, the high-rate system experiences more neutral variation than the low-rate system, because its direct contact with the influent results in a continuous inoculation by sewage micro-organisms, while the low-rate system lies downstream of the high-rate system and is, thus, buffered against direct inoculation from the sewage. This is in

concordance with the findings of (Gonzalez-Martinez et al., 2016), who sampled a number of AB-systems and showed that the high-rate systems share a core microbiome with their sewage influent, while this was not the case for the downstream low-rate systems. It should be noted that part of the unexplained variation in both datasets may have been an artifact caused by heterogeneity in the sludge when samples were taken, which may have caused some species to be overrepresented or underrepresented in a sample by chance. Because high-rate systems seem to have a larger fraction of unexplained variation, they may potentially be less controllable for technological applications. On the other hand, they may also be less sensitive to disturbances caused by environmental perturbations. The potentially lower controllability of high-rate communities may motivate the development of specialized control strategies, such as periodic re-inoculation with external sludge or the operation of parallel treatment straits, in order to counteract digression of the community structure and function. On the other hand, it should be further investigated as to what extent naturally occurring neutral variations may provide high-rate communities with a higher degree of resilience and adaptability to influent fluctuations.

3.3 Question 3

"Are community shifts in abundant and transitional sub-communities more deterministic than shifts in rare sub-communities?"

The threshold of abundance to distinguish between abundant and rare members has been arbitrarily set at values from 0.01 % to 1 % of the total community (Bagchi et al., 2015; Campbell et al., 2011; Kim et al., 2013; Pedros-Alio, 2012). For any given dataset, it is important to assess the impact of varying this threshold, because it may influence the results of further ecological analyses (Gobet et al., 2010). In this work, the threshold of distinction between abundant and rare community members was varied between 0.01% and 1% and the distribution between continuously abundant, transitional and continuously rare phylotypes in both datasets was evaluated (**Figure 2.6**).

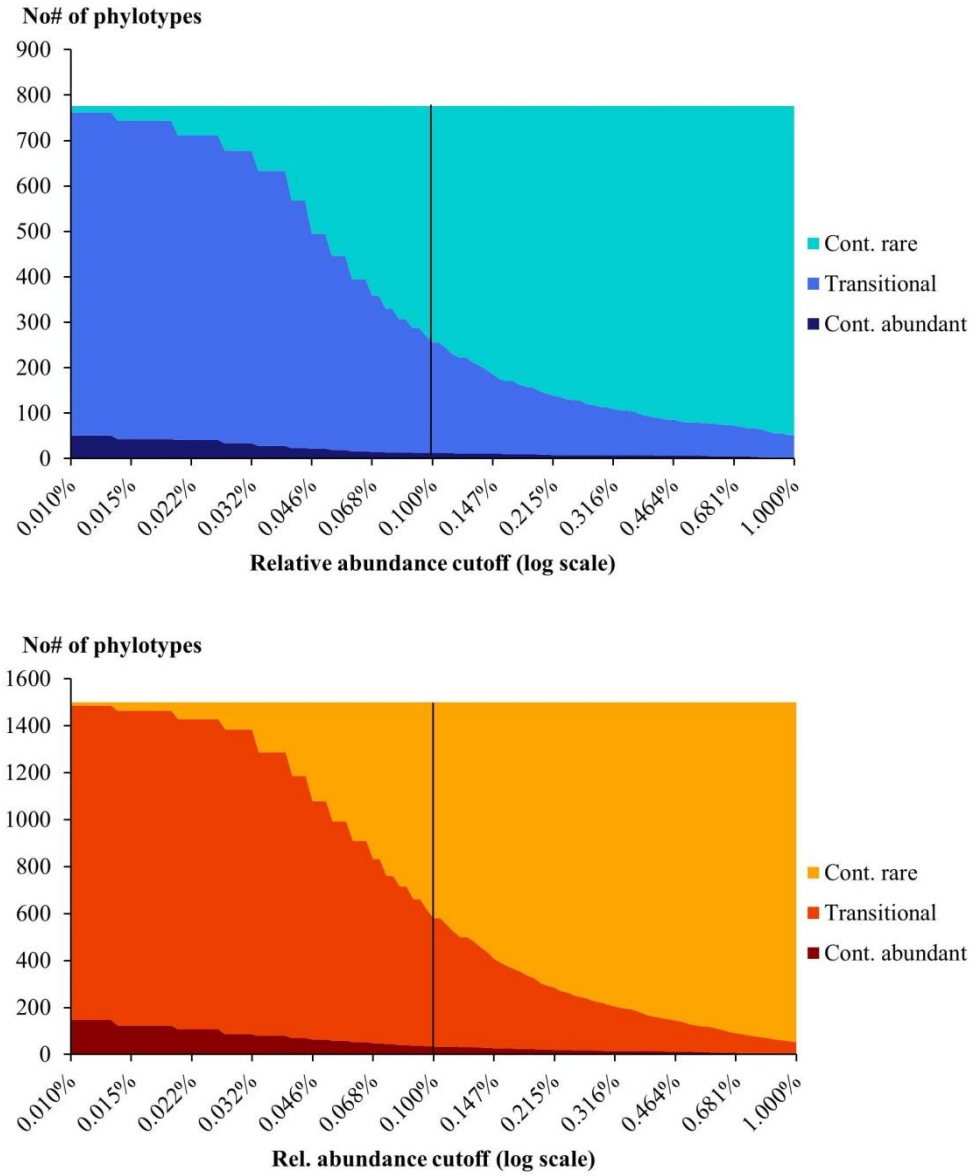


Figure 2.6: Distribution of continuously abundant, transitional and continuously rare members in the high-rate (top) and low-rate (bottom) communities, as a function of threshold of relative abundance.

A threshold of 0.1% relative abundance was considered to yield the most informative distribution: in the high-rate system, this threshold resulted in a continuously abundant sub-community of 1.7% of phylotypes and 60.7% of all sequences, and a continuously rare sub-community of 67% of phylotypes and 3.3% of sequences, with the remainder constituting the transitional sub-community. In the low-rate system, a similar distribution was obtained (**Table 2.3**).

Table 2.3: Distribution of phylotypes and sequences of the continuously abundant, transitional and continuously rare sub-communities over the entire time series (38 samples) of the high-rate and low-rate communities. At each time point, abundant and rare phylotypes were distinguished by a 0.1% relative abundance threshold.

	High-rate				**Low-rate**			
	Phylotypes		**Sequences**		**Phylotypes**		**Sequences**	
Continuously abundant	16	2.1%	3.8×10^5	65.2%	34	2.3%	2.5×10^5	43.2%
Transitional	237	30.5%	1.8×10^5	31.5%	547	36.5%	2.8×10^5	49.4%
Continuously rare	523	67.4%	1.9×10^4	3.3%	919	61.3%	4.3×10^4	7.4%
Total	776		5.8×10^5		1500		5.8×10^5	

The distribution of phyla differed along sub-communities. In all cases, Proteobacteria were dominant, followed by Bacteroidetes. In both the high-rate and the low-rate system, the continuously abundant sub-communities were nearly completely composed of Proteobacteria while the transitional sub-communities were near-equally dominated by Proteobacteria and Bacteroidetes. The continuously rare sub-communities were again dominated by Proteobacteria, followed by Bacteriodetes and a number of other phyla (**Figure 2.7**). A similar dominance of Proteobacteria and, to a lesser extent, Bacteroidetes was also reported in other studies that described phylogenetic distributions in abundant, transitional and/or rare sub-communities of activated sludge (Ibarbalz et al., 2014; Ju et al., 2014; Ju & Zhang, 2014; Kim et al., 2013; Saunders et al., 2016; Shade et al., 2014), and the dominance of Proteobacteria and Bacteroidetes has been observed in both high-rate and low-rate activated sludge communities (Gonzalez-Martinez et al., 2016). Still, significant differences were found for the relative abundance of Proteobacteria and Bacteroidetes between each of the sub-communities of the high-rate and low-rate system (p-value < 10^{-3} for each pairwise comparison). This suggests that these phyla play different functional roles in the system. For example, the lower relative abundance of Bacteroidetes in the abundant sub-communities compared to the transitional sub-communities raises the question whether species of this phylum are less likely to exert a core ecosystem function.

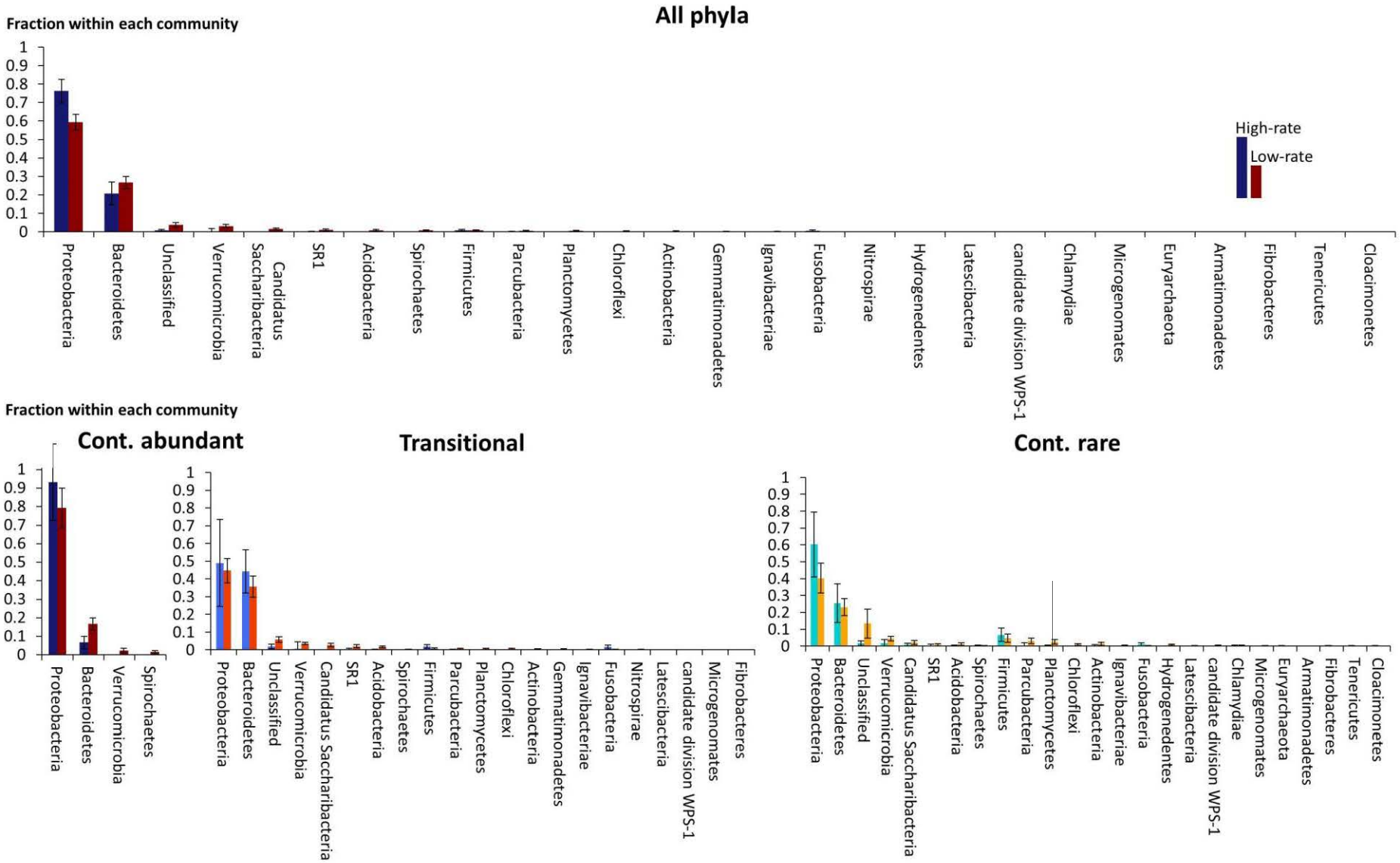


Figure 2.7: Distribution of phyla in the total community of the high-rate and low-rate systems (top), and distribution within the continuously abundant, transitional and continuously rare sub-communities (bottom). Relative abundances are normalized to 100% within each sub-community. Error bars show standard deviations.

From the assumed functional roles of the abundant and transitional sub-communities, it may be hypothesized that dynamic changes in these sub-communities are more deterministic than changes in the rare sub-community. A similar phenomenon has also been observed in macroecological studies, where the relative abundance of core species relied more on biological factors, while satellite species were more determined by random dispersal (Magurran & Henderson, 2003; Ulrich & Zalewski, 2006). Separate CA analyses for each sub-community of the high-rate and low-rate system were performed (**Figure 2.8**). Subsequent CCA analyses showed that, in both the high-rate and low-rate systems, larger fractions of community variation could be correlated to changes of environmental variables for the abundant and transitional sub-communities than for the continuously rare sub-communities (**Table 2.2**). The same trend was observed when different abundance thresholds were used to distinguish the sub-communities from one another. Indeed, as reviewed by Lynch and Neufeld (2015), previous studies on aquatic ecosystems have shown that rare sub-communities may be disproportionally influenced by random factors, but may retain a certain degree of activity and susceptibility to selective environmental factors. The results of this study support the theory that part of the rare community may act as a 'seed bank' waiting for the right growth conditions, and controlled by neutral factors.

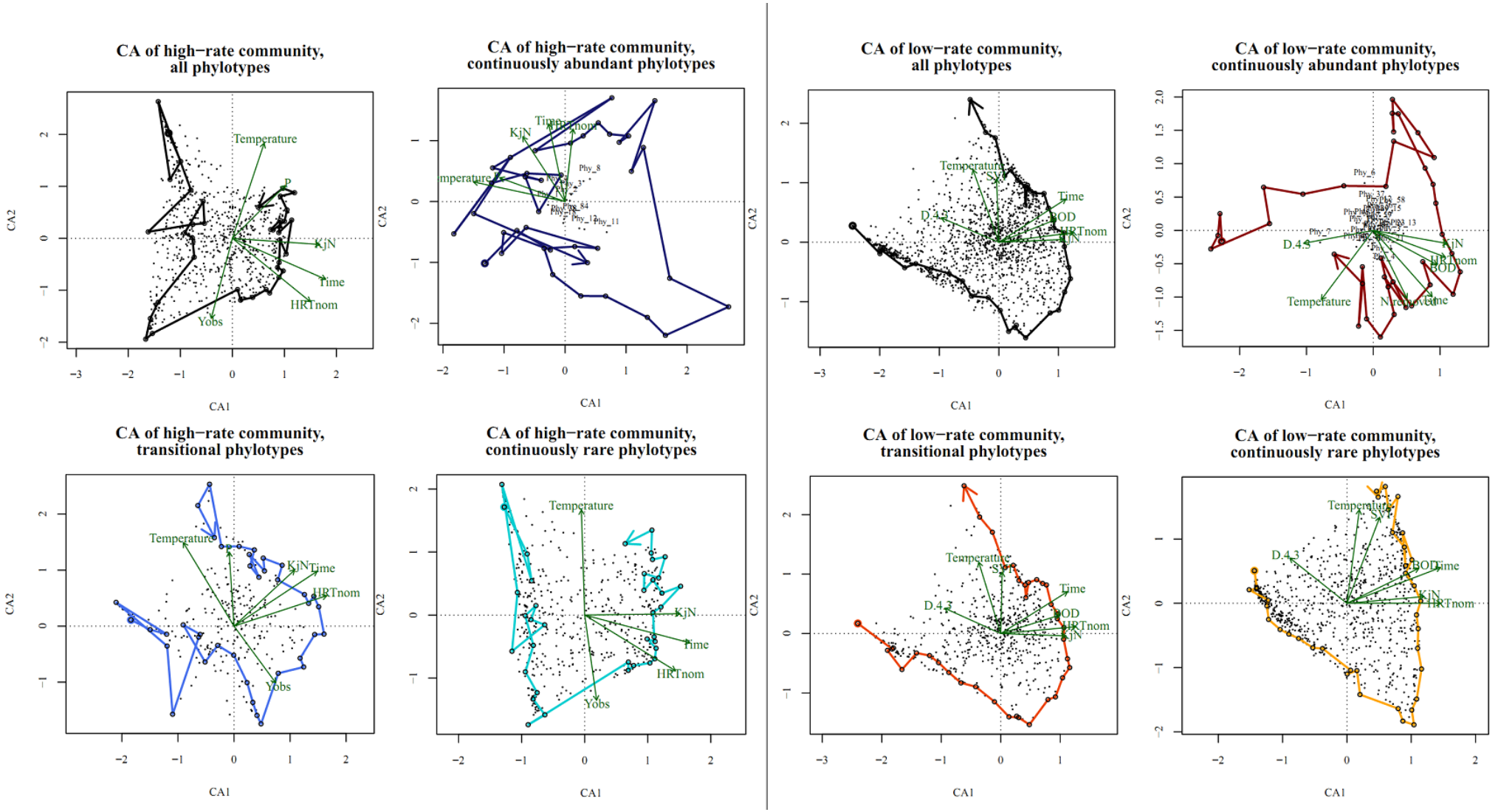


Figure 2.8: Correspondence analysis (CA) of the combined high-rate (blue) and low-rate (red) communities from October 2013 to July 2014. Phylotypes are shown as dots, samples are shown as circles connected by a colored arrow in chronological order. Environmental variables that significantly correlate to the ordination are plotted as green arrows.

3.4 Question 4

"Do high-rate community members show a lower co-occurrence and lower correlation with environmental variables than low-rate community members?"

Microbial co-occurrence may be direct (e.g., biological interactions) or indirect (e.g., shared ecological niches), but always reflect a deterministic relationship, rather than neutral association (Barberan et al., 2012). Co-occurrence networks of the high-rate and low-rate communities were created, based on pairwise Pearson correlations between phylotype abundances (**Figure 2.9**). The continuously rare sub-communities were excluded from the network analysis to filter out infrequent phylotypes, and to avoid that the network loses specificity due to low site similarities (Berry & Widder, 2014). After their exclusion from the datasets, the mean Jaccard similarity between sites was 49% for the high-rate system and 47% for the low-rate system, and thus higher than the minimum of 20% recommended by Berry and Widder (2014).

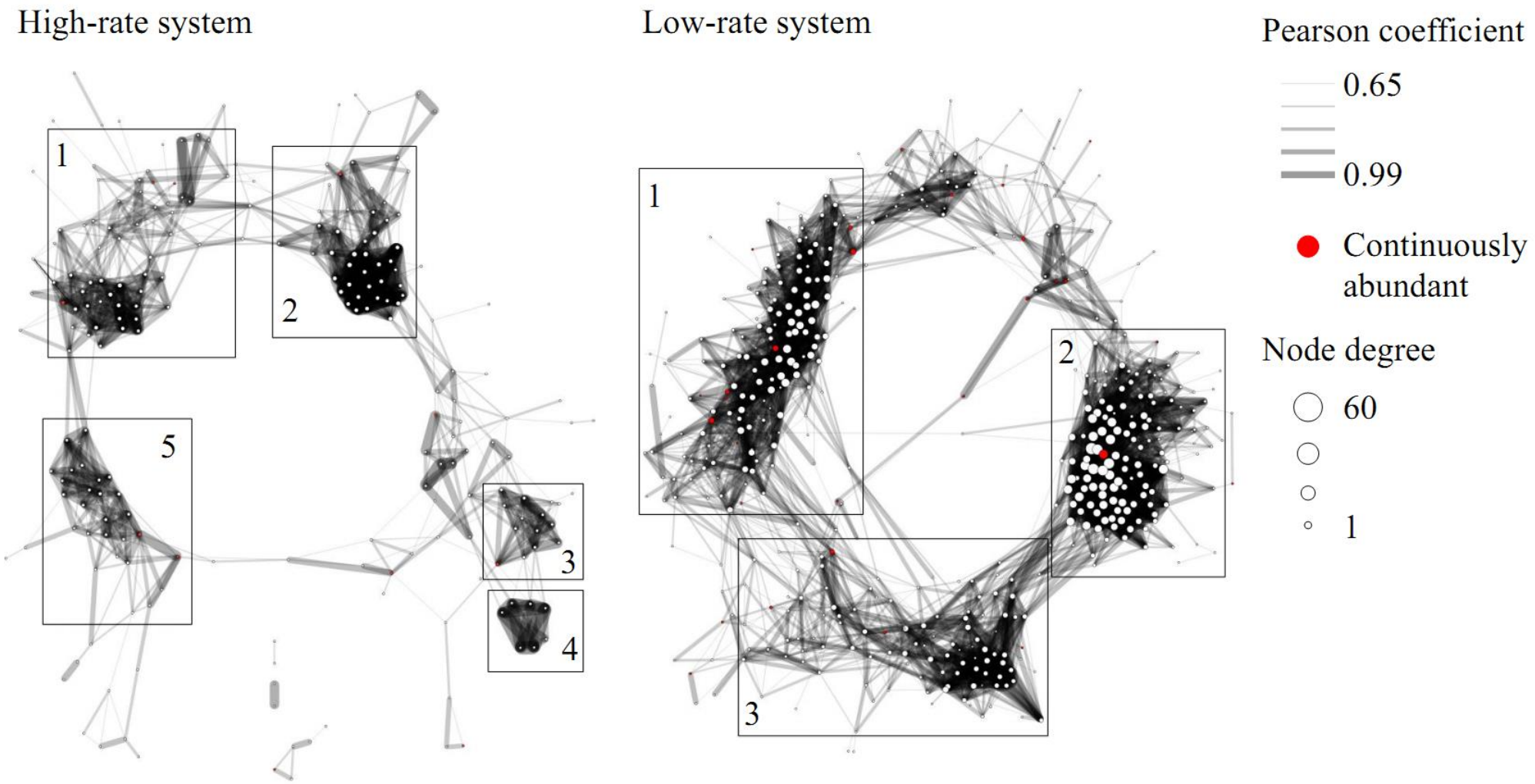


Figure 2.9: Co-occurrence network of the high-rate (left) and low-rate (right) communities, based on Pearson correlations. Positive correlations ($r > 0.65$, $p < 0.05$) were considered for the continuously abundant (red) and transitional (grey) sub-communities; continuously rare phylotypes were excluded from the analysis. Singleton nodes (i.e., nodes not connected to any other node) are not visualized. The node size represents the node degree, and the line thickness represents the strength of the correlation. Rectangles indicate different clusters within each network, as visually identified.

The average node degree – i.e., the average number of connections per node – was 9.4 in the high-rate network and 18.5 in the low-rate network. This means that both systems may be considered highly interconnected (Barberan et al., 2012). With 256 nodes, the high-rate network had 1203 edges, which constituted 3.7% of the total of 3.3×10^4 possible edges of a fully saturated network. The low-rate network had 581 phylotypes and 5,378 edges, which constituted 3.2% of the total of 1.7×10^5 possible edges. Therefore, when corrected for the number of network nodes, the high-rate and low-rate communities had a similar co-occurrence pattern. In the high-rate network, five loosely connected clusters of nodes could be distinguished, and in the low-rate network three. Throughout the study period, these clusters successively dominated their respective community in terms of abundance (**Figure 2.10**).

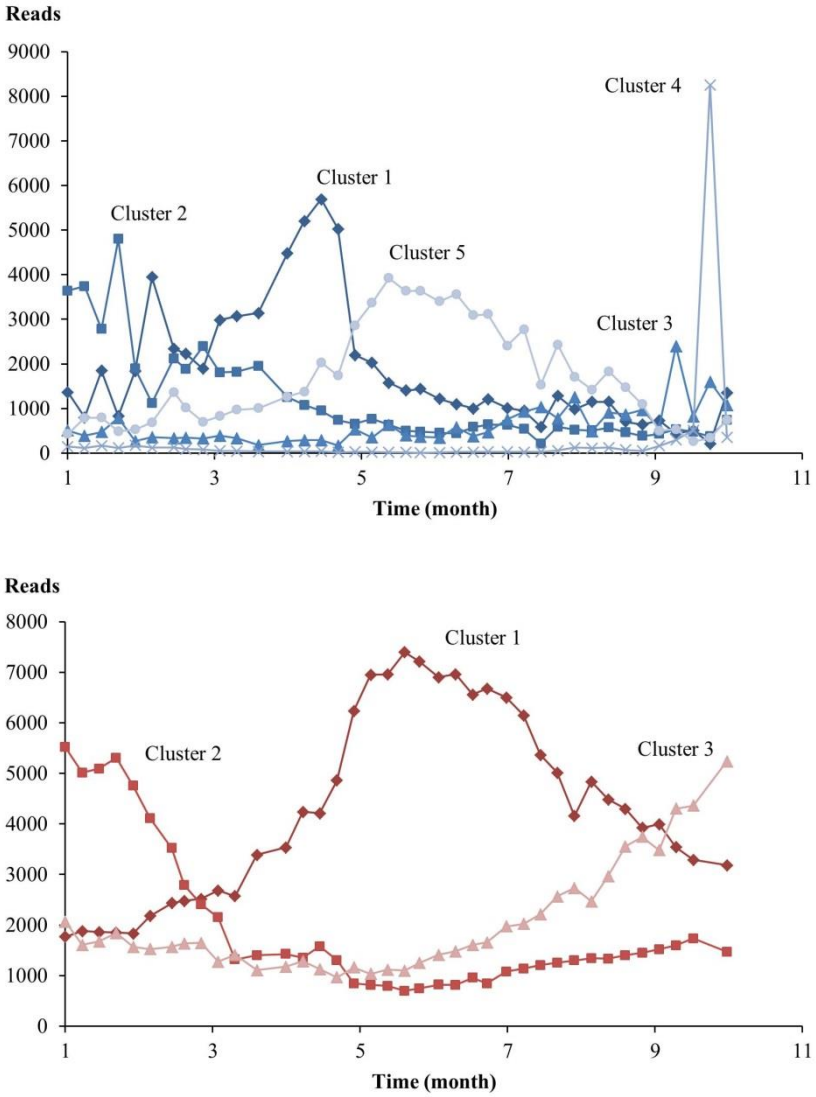


Figure 2.10: Evolution of absolute abundance of the five clusters in the high-rate network (top) and the three clusters in the low-rate network (bottom) throughout the study period. Cluster numbers are the same as in **Figure 2.9**.

Keystone community members are defined as having a disproportionately strong effect on their ecosystem functioning relative to their abundance (Paine, 1995). To identify keystone members from a co-occurrence network, the most likely candidates are nodes that are highly connected and centrally clustered, and can be indicated by network metrics, such as a high node degree, low betweenness centrality and high closeness centrality (Berry & Widder, 2014). Based on evaluation of these three parameters, the strongest keystone characteristics were found for Comamonadaceae gen. sp. (Phy 229), Bacteroidetes gen. sp. (Phy 208), SR1 gen. sp. (Phy 313) and *Rhodoferax* sp. (Phy 31) in the high-rate system (**Table A.I.1** in Annex I). *Rhodoferax* is known for its facultative photoheterotrophic and denitrifying metabolism (McIlroy et al., 2015), and showed a strong negative correlation with the HRT (r = -0.74) and KjN concentration (r = -0.72) in the high-rate system. In the low-rate system, the strongest keystone characteristics were found for *Sorangium* spp. (Phy 513, Phy 542 and Phy 245) (**Table A.I.2** in Annex I). These three phylotypes showed a negative correlation with the KjN concentration (r = -0.73 to -0.67). *Sorangium* is a genus of Myxobacteria with cellulose-degrading capabilities (Hou et al., 2006). No *Sorangium* sp. were detected in the high-rate system, which may be a result of their slow growth rate (Rachid et al., 2007). In both systems, all of the phylotypes with the strongest keystone characteristics belonged to the transitional sub-community, except for *Dokdonella* sp. (Phy 7), a keystone candidate in both systems, which was transitional in the high-rate system and continuously abundant in the low-rate system. *Dokdonella* is an aerobic heterotroph known for its presence in activated sludge (McIlroy et al., 2015). In the low-rate system, its abundance strongly correlated with temperature (r = 0.73). Certain phylotypes were continuously abundant but correlated neither with any other phylotype nor with any environmental variable included in this study. In the high-rate system, these included *Acidovorax* sp. (Phy 2), a genus of aerobic and denitrifying heterotrophic bacteria (McIlroy et al., 2015), and *Aquabacterium* sp. (Phy 12), a genus of microaerophilic denitrifying bacteria that may play a role in phosphorus removal (Kalmbach et al., 1999). In the low-rate system, these included Phy 2, *Sulfuritalea* sp. (Phy 14), a facultative autotrophic genus involved in sulfur and hydrogen oxidation (Kojima & Fukui, 2011), Sphingobacteriales gen. sp. (Phy 19), Chitinophagaceae gen. sp. (Phy 74), and *Derxia* sp. (Phy 101), a genus of facultatively autotrophic hydrogen oxidizers (Dworkin et al., 2006). It may be argued that the continuously abundant presence of these phylotypes through time suggests that their abundance is influenced by unidentified deterministic functional or environmental factors, rather than neutral assembly. Possibly, correlations were too weak to be detected in this work, but would be more pronounced if the dataset were expanded to include a broader range of values for the environmental and operational variables. On the other hand, previous research has demonstrated that some microorganisms may be abundant in activated sludge despite a low net growth-rate, due to the continuous influx of microorganisms with the sewage (Saunders et al., 2016).

To assess whether correlations with environmental variables are less strong in high-rate communities than in low-rate activated sludge communities, correlations between individual phylotypes and environmental variables were calculated, and the percentage of correlations exceeding a given threshold counted (**Figure 2.11**).

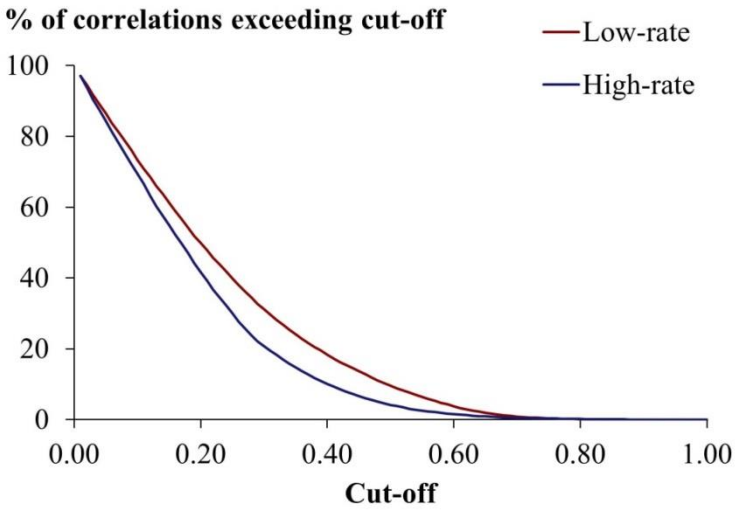


Figure 2.11: Percentage of all Spearman correlations between individual phylotypes and environmental variables for which the absolute coefficient exceeds a given value between r = 0 and r = 1, in the high-rate and low-rate community.

Between r = 0.45 and r = 0.7, the percentage of absolute correlation coefficients exceeding a given threshold in the high-rate community was always roughly half the fraction in the low-rate community. This indicates that community members in high-rate activated sludge are less likely to be correlated to environmental variables than in low-rate activated sludge. In the high-rate community, the strongest correlations were found with time (43 phylotypes with absolute correlation coefficient > 0.7), temperature (25 phylotypes) and KjN (5 phylotypes). In the low-rate community, these were time (135 phylotypes), temperature (46 phylotypes), nitrogen removal efficiency (22 phylotypes) and hydraulic retention time (11 phylotypes).

Overall, these results confirm that high-rate community members are less strongly correlated to environmental variables than members of low-rate activated sludge communities. This supports the hypothesis that high-rate communities are more subjected to neutral factors than low-rate communities, such as stronger oscillations in species abundances caused by the shorter SRT (Saikaly & Oerther, 2004), as presented in **Question 2**, or continuous random colonization by new species from the influent microbiome (Ofiţeru et al., 2010).

4 Conclusions

We investigated the microbial ecology of high-rate and low-rate activated sludge communities of a full-scale STP system, in terms of community structure, composition and sensitivity toward changes in environmental and operational variables. We showed that that:

- High-rate and low-rate communities are distinctly different in terms of richness, evenness and composition
- Both communities show a similar degree of weekly dynamics, but high-rate system dynamics are more variable
- High-rate communities are less shaped by deterministic factors, such as environmental and operational variables, than low-rate communities
- In both systems, continuously abundant and transitional sub-communities are more shaped by deterministic factors than the sub-community of continuously rare members
- High-rate community members show a co-occurrence pattern similar to that of low-rate community members, but are less likely to be correlated to environmental variables.

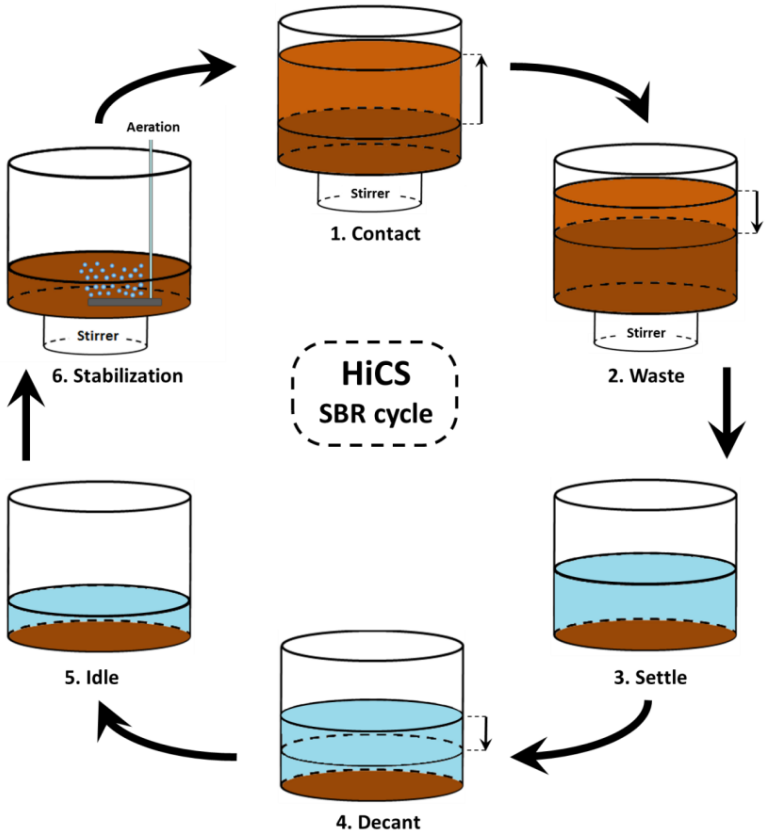
These findings provide a first basis for understanding how high-rate communities differ from conventional low-rate communities, and may facilitate a faster adoption of high-rate processes for improving the energy balance of sewage treatment plants. Differences in operational and environmental variables in a high-rate system result in a distinctly different microbial community compared to low-rate systems. This community differentiation may contribute to the improved overall performance of two-stage STPs in terms of energy and resource recovery. Additionally, the relatively high importance of neutral factors in shaping the community of high-rate systems suggest that they may be less sensitive towards external shocks and perturbations, but at the same time be more challenging to steer by controlling the operational conditions. Future studies should assess the implications for process engineering of high-rate systems, in order to develop specialized optimization and control strategies.

5 Acknowledgements

F.A.M and S.E.V were supported, respectively, as doctoral candidate (Aspirant) and postdoctoral fellow by the Research Foundation Flanders (FWO-Vlaanderen). H.R. was supported by the European Research Council (ERC) starter grant ELECTROTALK. D.S. was supported by the Agency for Innovation by Science and Technology in Flanders (IWT). R.V.V. and N.B. were supported by the Inter-University Attraction Pole (IUAP) 'μ-manager' funded by the Belgian Science Policy (BELSPO, P7/25). We thank Arne Verliefde and Quenten Denon for assistance with the particle size measurements, Hans Mollen, Leonie Hartog, Etteke Wypkema and Waterschap Brabantse Delta for supplying plant data and allowing the use of the plant facilities, Harry Vrins and Antoon Buijnsters for assistance with sampling, Bert Bundervoet and Colsen NV for practical assistance and logistics, Iris Plumeier and Silke Kahl for assistance with the sequencing, Frederiek-Maarten Kerckhof and Stijn Decubber for assistance with the analyses, and Jo De Vrieze and Ruben Props for the inspiring scientific discussions.

CHAPTER 3:

CAN HIGH-RATE CONTACT STABILIZATION (HICS) SUBSTITUTE A HIGH-RATE CONVENTIONAL (HICAS) SYSTEM?



Cover figure: HiCS SBR cycle (Koen Pauwels).

This chapter has been redrafted after:

MEERBURG, F. A., BOON, N., VAN WINCKEL, T., VERCAMER, J. A. R., NOPENS, I. & VLAEMINCK, S. E. (2015). Toward energy-neutral wastewater treatment: A high-rate contact stabilization process to maximally recover sewage organics. *Bioresource Technology* **179**: 373-381.

1 Introduction

To advance toward energy-positive wastewater treatment, it is necessary to maximize the capture of organic matter, and thus increase the relative contribution of sorption and storage of substrates rather than oxidation or extensive bioconversion processes. We theorize that a high-rate contact stabilization (HiCS) system may be more efficient than the high-rate conventional HRAS system (HiCAS) in recovering chemical energy as biogas. A HiCS process may combine the advantages of high SLR and low SRT (similar to the HiCAS process) with a selective pressure toward sorption and storage (similar to the CS process).

A small number of studies have been performed on activated sludge processes that can tentatively be identified as HiCS processes. Huang and Li (2000) found that a high-rate system in contact stabilization configuration achieves rapid adsorption of substrates during the contact phase, and Zhao et al. (2000) suggested that such a system may perform better than HiCAS in terms of substrate removal. However, a systematic comparison is needed to evaluate the performance of the HiCS system in terms of energy recovery and their perspectives toward implementation in an energy-neutral wastewater treatment scheme.

We operated a HiCS system for comparison with HiCAS, CAS and CS systems at laboratory scale. **Figure 3.1** compares the reactor configuration of the CAS and CS systems and their high-rate variants, the HiCAS and HiCS system. The HiCS system was operated at two different ratios of contact to stabilization time (t_c:t_s) and the HiCAS system was operated at two different SRTs to evaluate the importance of these design parameters. Here, we describe the reactor performances in terms of organic matter removal, sludge production and biogas production during anaerobic digestion of the produced sludge. Additional experiments were performed to elucidate process kinetics and explore the potential for further optimization of the HiCS system.

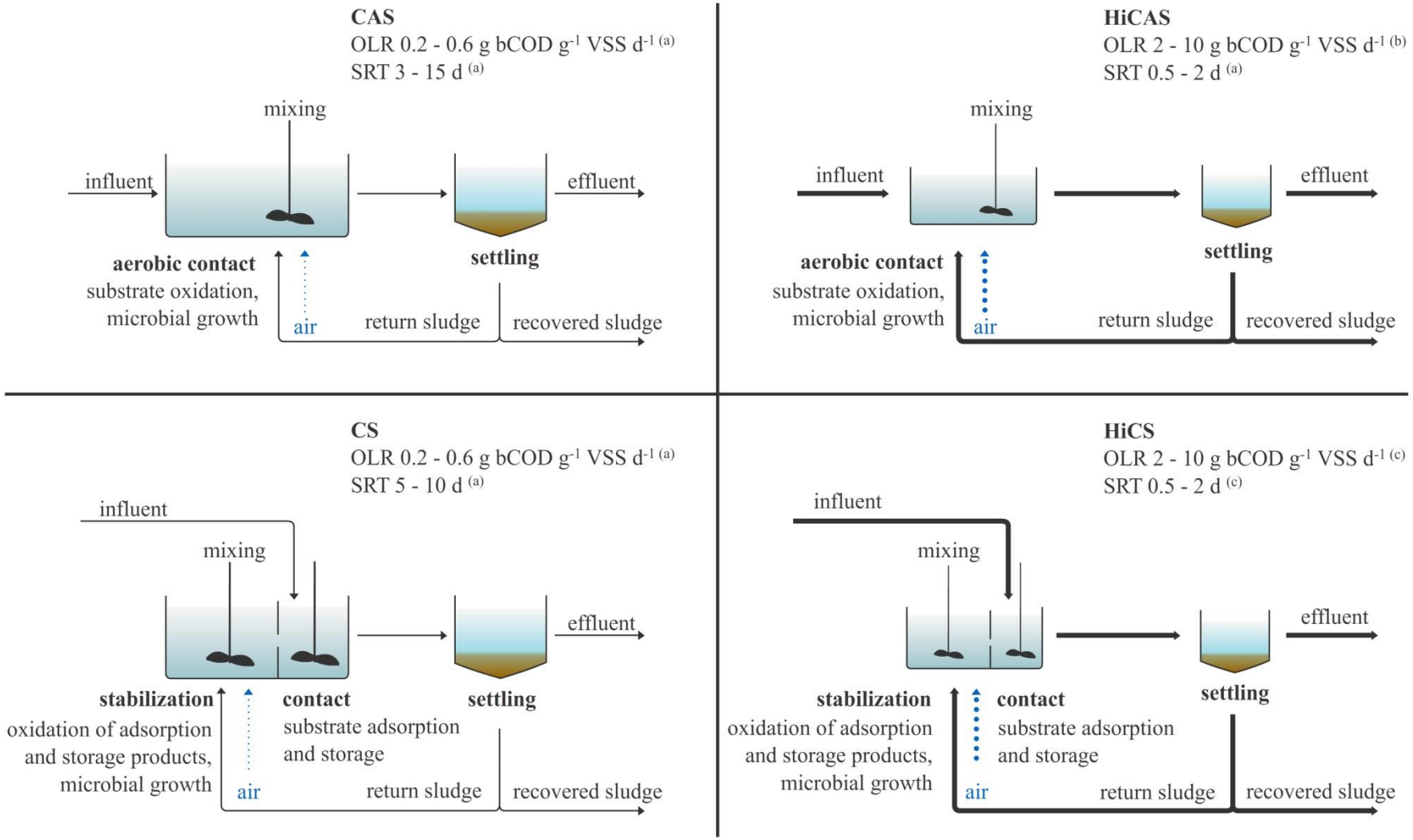


Figure 3.1: Schematic representation of the reactor configurations of the conventional activated sludge (CAS), contact stabilization (CS), high-rate conventional activated sludge (HiCAS) and high-rate contact stabilization (HiCS) systems, together with recommended values of sludge-specific loading rate (organic loading rate, OLR) and sludge retention time (SRT). Differences in flow rates are qualitatively represented by arrow thickness. [(a)] Metcalf & Eddy (2003); [(b)] Böhnke et al. (1997c); [(c)] this work.

2 Material and methods

2.1 Reactor operation

The CAS, CS, two HiCAS and two HiCS reactors were operated as sequencing batch reactor (SBR) with a volume exchange ratio of 50% and an influent flow rate of 4 L d^{-1} for the CAS and CS reactors and 24 L d^{-1} for the HiCAS and HiCS reactors, to make sure the systems conformed to the definition of high-rate systems, with a minimal SLR of 2 g bCOD g^{-1} VSS d^{-1} and a maximal SRT of 2 d (see Chapter 1). The influent consisted of synthetic wastewater, and was prepared as described by Aiyuk and Verstraete (2004). Influent characteristics are listed in **Table 3.1**. The working volume of each reactor was 2 L during the react phase and 1 L during the stabilization phase. The duration of the reactor phases is given in **Table 3.2**. As such, the HiCS reactors were operated at a different ratio of contact to stabilization time (t_c:t_s). The values for the contact time were chosen based on (Böhnke et al., 1997b) and Wahlberg et al. (1994), who demonstrated that bioflocculation processes occur mainly within the first 10 minutes of reaction time. The values for the stabilization time were chosen based on Huang and Li (2000), who found that at least 30 minutes of stabilization is required in a HiCS system.

Table 3.1: Measured influent characteristics of the synthetic wastewater and diluted black water. Standard errors are shown behind values.

	COD_{tot} mg L^{-1}	**COD_{diss}** mg L^{-1}	**BOD_5** mg L^{-1}	**TSS** mg L^{-1}	**VSS** mg L^{-1}
Synthetic wastewater					
High-rate reactors	757 ± 41	489 ± 23	460 ± 34	206 ± 34	184 ± 30
Low-rate reactors	492 ± 46	353 ± 36	460 ± 34	116 ± 14	116 ± 11
Diluted black water	270 ± 17	160 ± 9	139 ± 16	56 ± 6	51 ± 4

Table 3.2: Duration of the different phases of the SBR reactors. n.a.: not applicable. A visual representation of each phase is shown in the cover figure of this chapter.

Reactor	**SRT**	**t_c:t_s**	**Stabilization**	**Contact**		**Settling**	**Withdrawal**	**Idle**
				Fill and react	React			
	d		min	min	min	min	min	min
CAS	10	n.a.	n.a.	270	45	30	5	10
CS	12	30:285	285	25	5	30	5	10
HiCAS	1.31	n.a.	n.a.	30	10	15	3	2
	0.41	n.a.	n.a.	30	10	15	3	2
HiCS	1	20:20	20	17	3	15	3	2
	1.1	5:35	35	4	1	15	3	2

In additional experiments, a HiCAS and a HiCS reactor were operated as continuous stirred-tank reactor (CSTR) with a recycle ratio of 160%. The influent consisted of diluted black water to evaluate the extrapolation of obtained results with high-strength synthetic wastewater to real wastewater with a lower strength and different composition. The black water was stored at 4°C for a maximum period of 2 weeks. Before use, it was sieved (1 mm) and diluted with tap water to match the values listed in **Table 3.1**. The HiCAS system consisted of a 3 L contactor and 1 L settler; the HiCS system consisted of a 1 L contactor, a 1 L settler and a 2 L stabilizer reactor. The influent flow rate was 2.9 L h^{-1} to obtain a contact time of 24 minutes in the HiCAS reactor and a contact time of 8 minutes and a stabilization time of 26 minutes in the HiCS reactor.

The high-rate as well as the low-rate reactors were operated without preceding treatment of the raw influent. Aeration was performed during the contact phase of all CAS and HiCAS systems and during the stabilization phase of all CS and HiCS systems. Stirring was performed in both the contact and stabilization phases. In the aerated phases, the dissolved oxygen (DO) concentration was kept above 2 mg L^{-1}. Sludge wasting was performed during the react phase in the SBR reactors and from the return stream in the continuous reactors. The waste flow rate was adjusted at least three times per week to maintain the desired sludge retention time (SRT). Samples were taken three times a week during steady-state operation of the reactors, which was typically reached after an acclimation period of at least two days. The operational details of the reactors at steady-state are shown in **Table 3.3**.

All reactors were operated in a temperature-controlled room at 15°C to mimic average wastewater temperatures of Western Europe, and automatically controlled at a pH between 7.7 and 8.3 (Consort, Belgium). Overhead stirring (propeller type, diameter 7 cm, 100-150 rpm), peristaltic pumping and aeration (air flow rate 1.5 - 2 L min^{-1} during aerated phases) were performed mechanically. Reactors were inoculated with fresh HiCAS sludge from the full-scale wastewater treatment installation of Nieuwveer (Breda, NL).

Table 3.3: Overview of reactor operation and sludge characteristics, at steady-state operation. n.a.: not applicable. Standard errors are shown behind values, where applicable.

	SRT_{tot}	**SRT_{aer}**	**t_c:t_s**	**Influent**		**Reactor type**	**Steady-state operation**	**Organic loading rate**	**Contactor VSS**	**VSS/TSS ratio sludge**	**COD_{part}/TSS ratio sludge**
	d	d	min:min	Synthetic	Diluted black water		d	g bCOD g^{-1} VSS d^{-1}	g L^{-1}	-	g COD_{part} g^{-1} TSS
CAS	10	8.9	n.a.	x		SBR	18	0.77 ± 0.07	1.64 ± 0.17	0.77 ± 0.03	1.23
CS	12	9.9	30:285	x		SBR	18	0.62 ± 0.07	1.77 ± 0.18	0.85 ± 0.02	1.15
HiCAS	1.31	0.87	n.a.	x		SBR	16	4.53 ± 0.58	2.21 ± 0.25	0.89 ± 0.02	1.34
	1.1	0.80	n.a.		x	CSTR	41	4.30 ± 0.67	2.33 ± 0.56	0.94 ± 0.01	1.37
	0.41	0.27	n.a.	x		SBR	16	12.5 ± 1.93	1.45 ± 0.51	0.88 ± 0.05	1.23
HiCS	1	0.33	20:20	x		SBR	15	6.23 ± 0.75	1.67 ± 0.16	0.96 ± 0.04	1.35
	1.2	0.94	8:26		x	CSTR	41	5.72 ± 0.92	1.28 ± 0.33	0.92 ± 0.04	1.43
	1.1	0.62	5:35	x		SBR	15	2.96 ± 0.23	3.32 ± 0.31	0.89 ± 0.03	1.66

2.2 Biochemical methane potential

Biochemical methane potential (BMP) experiments were performed to determine the amount of biogas production by means of anaerobic digestion (AD) of the sludge. The tests were performed in batch reactors with a total volume of 120 mL. The inoculum consisted of mesophilic AD sludge from a lab-scale reactor that was operated as a CSTR with semi-continuous feeding at 34°C (Innolab, Belgium) for the HiCAS sludge and granular mesophilic sludge from an upflow anaerobic sludge blanket reactor (UASB) treating brewery wastewater (Van Steenberge, Belgium) for the CAS, CS and HiCS sludge and positive control. Chemical characteristics of the anaerobic inocula are given in **Table A.II.1** of Annex II. The inocula were stored at 4°C and degased at 34°C for 24 hours before use. The substrate loading ratio was 0.3 g chemical oxygen demand (COD) g^{-1} $VSS_{inoculum}$. Dilutions were made with tap water until each reactor contained 80 mL of test solution at an inoculum concentration of 10 g VSS L^{-1}. The pH was uncorrected and remained between 7 and 8.5 throughout the experiments. The reactors were sealed at a slight underpressure and shaken under mesophilic conditions (34°C). The pressure increase in the headspace was measured with a gas pressure tensiometer (UMS, Germany), and the gas composition of the biogas was analyzed at the end of the experiment. Experiments were terminated when the biogas production curves reached a plateau (**Figure A.II.1** of Annex II), which was 71 days for the HiCAS sludge and 26-34 days for the other treatments. Negative controls without substrate were included to correct for residual methane production, and a positive control with glucose as substrate was added to verify the activity of the inoculum. All experiments and controls were performed in triplicate. Specific methane yield was expressed as the amount of methane COD ($COD_{methane}$) produced per gram of sludge TS fed.

2.3 Specific oxygen uptake rate

Respirometric experiments were performed in a 2 L reactor with temperature control at 15°C. The reactor setup was as described by Gernaey et al. (2002). The DO and pH (Mettler Toledo, Switzerland) were logged every second with Labview software (National Instruments, USA). The sludge-specific oxygen uptake rate (SOUR) of the sludge of the HiCS systems at t_c:t_s 20:20 and 5:35, as well as concentrations of colloidal and dissolved substrate, were monitored during three consecutive SBR cycles. Operational parameters of the SBR cycles were identical to those of the main experiments described in Section 2.1. Prior to the respirometric experiments, the sludge was aerated overnight to reach an endogenous state, and to determine the endogenous respiration rate (OUR_{endo}). The volumetric gas/liquid mass transfer coefficient (k_La) was determined from three consecutive re-aeration curves as described by Bandyopadhyay et al. (2009). The exogenous respiration rate was calculated as

$$OUR_{exo} = k_La(DO^{eq} - DO) - dDO/dt \qquad \text{Equation 3.1}$$

where DO^{eq} is the equilibrium DO concentration that is reached under aeration when only endogenous respiration occurs and dDO/dt is the change in the DO level over time. Through division by the sludge concentration, the exogenous SOUR ($SOUR_{exo}$) was calculated.

2.4 Analytical procedures

COD was measured using Nanocolor test kits (Macherey-Nagel, Germany) according to the manufacturer's instructions. Five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), volatile solids (VS) and total ammonia nitrogen were determined according to standard methods (Greenberg et al., 1992). bCOD was calculated as BOD_5 divided by 65%. To determine the fractions of total (COD_{tot}), particulate (COD_{part}), colloidal (COD_{coll}) and dissolved (COD_{diss}) substrate, samples were filtered over a 1.5 µm filter (Grade 934-AH, Whatman, UK) to retain particles, and a 0.45 µm filter (type PA-45/25, Macherey-Nagel, Germany) to retain colloids. Handheld meters were used to measure DO (Hach, USA), and conductivity and pH (Consort, Belgium). Biogas composition was analyzed with a Compact GC (Global Analyser Solutions, The Netherlands) equipped with two parallel channels: a first channel operated at 60 °C with a Porabond Q 2m x 0.32mm precolumn and a Molsieve 5A 30m x 0.32mm column for the detection of H_2, and a double second channel operated at 50 °C, with a first loop consisting of a Porabond Q 2m x 0.32mm precolumn and a Molsieve 5A 7m x 0.32mm column for determination of O_2, N_2 and CH_4, and a second loop consisting of an Rt-QS-Bond 2m x 0.32mm precolumn and an Rt-QS-Bond 28m x 0.32mm column for determination of CO_2. Both channels had a thermal conductivity detector operated at a temperature of 80 °C. For H_2 detection, the carrier gas was N_2 at a flow rate of 47.18 mL min^{-1}. For O_2, N_2 and CH_4 detection, and detection of CO_2, the carrier gas was He at flow rates of 36.08 mL min^{-1} and 10.82 mL min^{-1}, respectively. The lower detection limit for all gases was 1 ppmv.

2.5 Calculations

The SRT or sludge age was calculated over the entire reactor and settler system for all reactors. The removal performance of organic matter was addressed in terms of COD, to take into account removal of both biodegradable and non-biodegradable substrate by the different removal mechanisms involved. The COD balance was calculated from the COD fractions leaving the reactor system as recovered sludge (i.e., sludge produced by wasting, sampling and buildup in the reactor), effluent COD_{part} and effluent COD_{diss}, summed cumulatively over the entire period of steady-state operation. Fractions were expressed as percentage of influent COD_{tot}. All COD unaccounted for (i.e., entering but not leaving the system) was assumed to be lost by respiration to CO_2. The observed sludge yield was calculated as the daily sludge production (i.e., the amount of sludge produced by wasting, sampling, washout into the effluent and buildup in the reactor) divided by the daily substrate removal (i.e., the difference between COD_{tot} in the influent and COD_{diss} in the effluent). Thus, it was assumed that particulate matter leaving the reactor was sludge, not substrate. Removal percentages of dissolved and colloidal substrate in each reactor phase were determined from the substrate concentration profiles within each phase (for stabilization, settling and withdrawal) and by comparing actual substrate concentrations to the expected concentrations assuming only incoming substrate and no uptake or degradation (for the contact phase).

2.6 Statistical analyses

Normality of data residuals was tested using the Shapiro-Wilk normality test and homogeneity of variances using Levene's test. In cases where the null hypothesis of normality was rejected (i.e., for COD_{tot} removal rates and sludge yields), multiple comparisons were performed using the non-parametric Kruskal-Wallis rank sum test and post-hoc pairwise comparisons using the Mann-Whitney *U* test. In other cases (i.e., for COD_{diss} removal rates), multiple comparisons were performed using the parametric analysis of variance (ANOVA) test and post-hoc pairwise comparisons using the student's *t*-test. Pairwise comparisons were only performed if the null hypothesis of equal means was rejected, and *p*-values were adjusted using the Benjamini-Hochberg correction. Calculated parameters, for which means and standard deviations but no individual data points were available, were compared using the student's *t*-test for equal sample sizes and equal variances (specific methane yield) or unequal sample sizes and equal variances (energy recovery), using the Benjamini-Hochberg correction in all cases. Differences were considered significant at $p < 0.05$. All statistical analyses were performed with the software R version 3.0.2 for Windows (R Core Team, 2013).

3 Results and Discussion

Any treatment scheme that aims to recover energy as a resource from wastewater should aim to (1) minimize oxidation to CO_2 to avoid unnecessary loss of energy-containing organic matter, (2) concentrate particulate, colloidal and dissolved organic matter into a sludge stream to facilitate subsequent conversion of energy into useful forms and (3) minimize plant-wide energy consumption by implying energy-efficient technologies with close process control throughout the treatment train. This chapter addresses the first two criteria by comparing the chemical energy recovery potential of two configurations for high-rate activated sludge – the better known HiCAS and the more novel HiCS configuration – as primary treatment processes for domestic sewage.

3.1 Reactor operation and organic matter removal rates

COD removal rates were always significantly lower in the low-rate reactors than in their respective high-rate counterparts ($p < 0.05$). However, removal rates did not differ significantly among high-rate reactors, with the exception of the relatively poor removal rate of COD_{diss} in the HiCS reactor at t_c:t_s 20:20 (**Figure 3.2**).

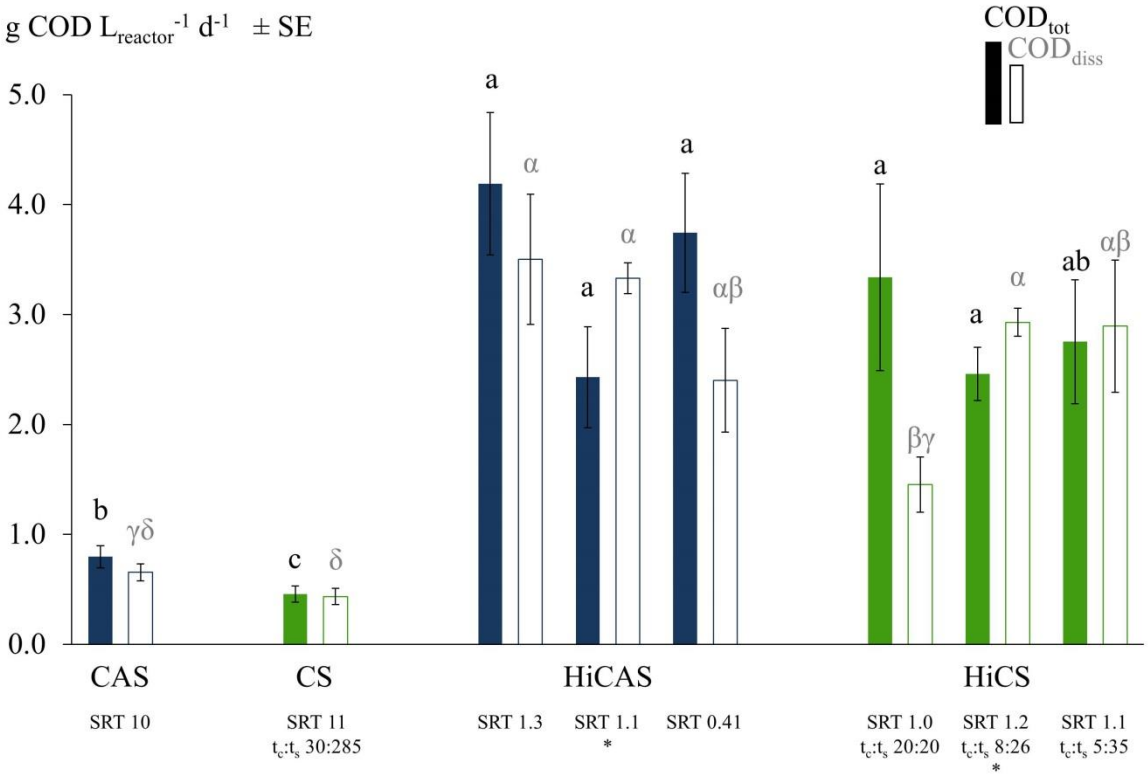


Figure 3.2: COD removal rates of the different reactors. Error bars show standard errors (SE). Reactors indicated with asterisk (*) were operated on diluted black water in CSTR mode; all other reactors were operated on synthetic wastewater in SBR mode. Significance groups for pairwise comparisons are shown for COD_{tot} (Roman letters) and COD_{diss} (Greek letters) separately; if two bars share the same letter, they do not differ significantly ($p > 0.05$).

Within none of the reactor runs, significant differences could be observed between COD_{tot} and COD_{diss} removal rates, indicating that removal of dissolved COD had a major influence on overall removal efficiencies. No differences in removal rates were detected between reactors treating synthetic wastewater and reactors treating diluted black water. This suggests that differences in their respective influent compositions were not of major influence on the physical and biological phenomena investigated here, and that results obtained with high-strength synthetic wastewater may be extrapolated to low-strength real wastewater.

High COD removal rates in high-rate reactors are partly a consequence of the high SLRs applied. On the other hand, removal efficiencies were relatively low (40-55%; **Table 3.4**). This may be due to the low SRT and short hydraulic retention times, which cause a larger fraction of substrate to be non-degradable by high-rate sludge compared to low-rate sludge (Haider et al., 2003). Any residual COD in the effluent of a high-rate activated sludge system may be removed aerobically or anoxically in subsequent secondary treatment processes for nitrogen removal (De Clippeleir et al., 2013b).

However, in light of energy sustainability, the efficiency of the solid/liquid separation should be improved to maximize the removal and recovery of COD. Removal efficiencies of inorganic nitrogen and phosphorus species in the high-rate reactors were low to negligible (data not shown), and their optimization was considered outside the scope of this research, given the compatibility of high-rate activated sludge processes with downstream secondary treatments for nutrient management. Future work should assess differences in BOD and COD removal, since removal of organic matter by adsorption and flocculation may differ between biodegradable and non-biodegradable substrate. Moreover, the residual biodegradable organic matter in the high-rate stage effluent will influence the performance of secondary treatment options, such as nitrogen removal with partial nitritation/anammox.

3.2 Carbon fractionation and conversion

To determine the fate of the removed substrate, an overall COD balance was made of the steady-state phases of the different reactors (**Figure 3.3**).

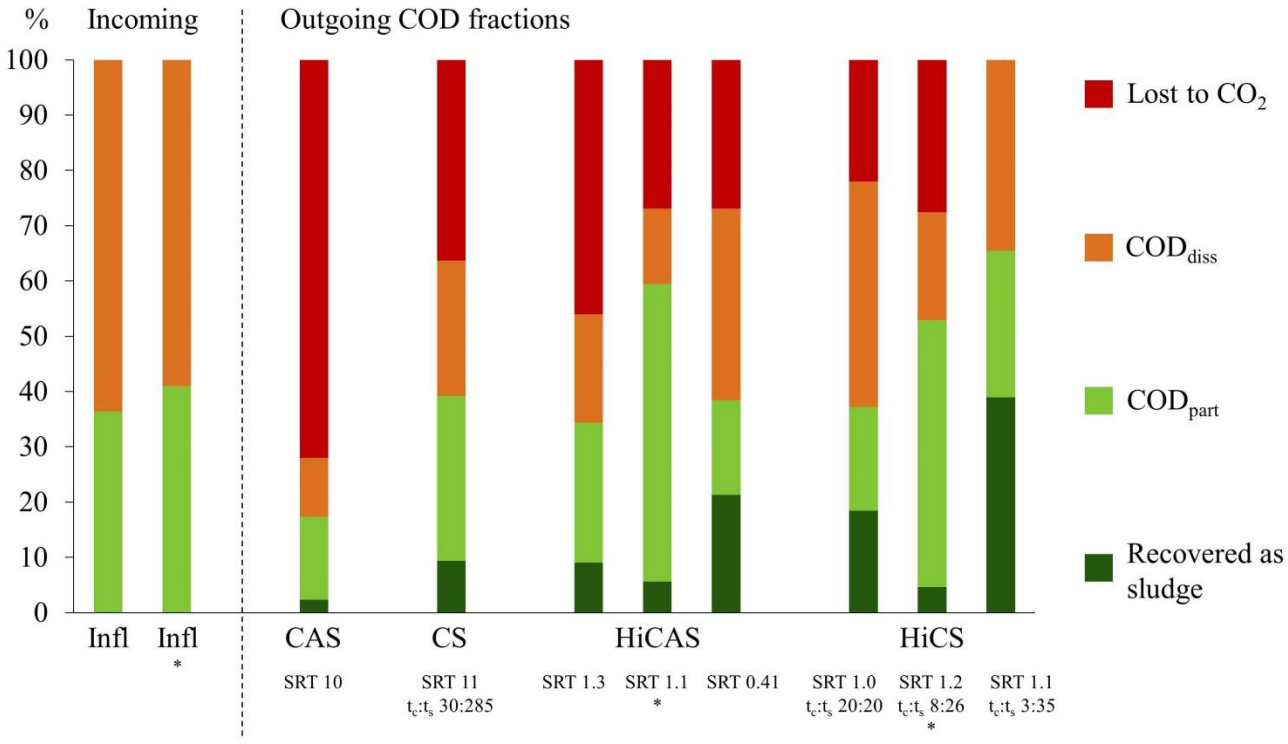


Figure 3.3: Cumulative COD balance over the entire steady-state period of the experiments, showing (left) incoming fractions of COD_{diss} and COD_{part} in the synthetic wastewater and diluted black water, and (right) the fate of COD in the different reactors, as fractions recovered as sludge, effluent COD_{part}, effluent COD_{diss} and lost to CO_2. Reactors indicated with asterisk (*) were operated on diluted black water in CSTR mode; all other reactors were operated on synthetic wastewater in SBR mode.

The extent of substrate oxidation (i.e., the cumulative fraction lost to CO_2) was lowest in the HiCS reactors and even approximated zero for the reactor at t_c:t_s 5:35. This indicates that the extent of oxidation was very limited in the latter reactor, although substrate oxidation was still detected by means of SOUR measurements (**Figure 3.5**). The cumulative percentage of removed COD (i.e., the sum of the fractions going to recovered sludge and CO_2) was highest in the CAS reactor (74%) and lowest in the HiCAS (32% to 55%) and HiCS reactors (32% to 40%). In contrast, the percentages of COD recovered as sludge were highest in the HiCS reactor at t_c:t_s 5:35 (40%). Due to non-optimized solid/liquid separation in the settlers, particulate fractions in the effluent were still relatively high in all reactors, most notably the reactors operated on diluted black water (48% of incoming COD leaving as COD_{part} for HiCS and 54% for HiCAS). Throughout the experiments, variable and high sludge volume indices were observed (between 50 and over 300 mL/g). This is typical for sludge grown at high SLR and short SRT (Liao et al., 2006; Ramalho, 1977a).

A high washout of COD_{part} in the reactors operated on diluted black water was responsible for their overall worse COD removal and recovery performance compared to the high-rate reactors on synthetic wastewater, because both reactors on diluted black water achieved COD_{diss} removal percentages at least as high as their counterparts on synthetic wastewater. With an average influent flow rate of 2.9 L h^{-1} and a surface area of 0.015 m^2, the upflow velocity in the settler was 0.19 m h^{-1}. This should be sufficiently low to allow settling of particles, since it is below the range of 1.3 – 3 m h^{-1} recommended for primary treatment stages with sludge return (Metcalf & Eddy, 2003). By comparison, the high-rate SBR reactors on synthetic wastewater had an approximate upflow velocity of 0.4 m h^{-1}, because of the approximate travel distance of 10 cm during the 15-minute settling phase. These differences in upflow velocity are contrary to the fact that the SBR reactors on synthetic wastewater achieved a better solid/liquid separation. Possibly, the low strength and lower biodegradability of the diluted black water had a negative influence on the settleability of sludge and/or particulate COD. When high-rate systems experience prolonged problems with settleability, batch experiments may be performed to construct settling velocity curves and determine characteristics such as particle size distributions, thresholds of flocculation and initial settling classes (see Chapter 6), in order to determine which mechanism in the coagulation - flocculation - settling process is a performance-limiting step. Eventually, alternatives to simple gravitational settling may be explored, such as chemically enhanced settling by means of dosing coagulants and/or flocculants, membrane filtration or dissolved air flotation.

The influent consisted of 36% COD_{part} in the synthetic and 41% COD_{part} in the diluted black water. Theoretically, perfect physicochemical solid/liquid separation of the raw influent would therefore allow an overall COD recovery of only 36% and 41%, respectively. This illustrates the advantage of high-rate biological processes over purely physicochemical primary treatment technologies, such as CEPT. The HiCS reactor at t_c:t_s 5:35 achieved a COD_{diss} removal of 46%. Therefore, assuming optimal solid/liquid separation and thus adding the fraction of effluent COD_{part} to the fraction of recovered COD, the HiCS reactor at t_c:t_s 5:35 would theoretically reach 66% COD recovery. Similarly, the other high-rate reactors would reach a recovery of 35-54% while the low-rate reactors would reach only

17-32%. This demonstrates the potential of high-rate activated sludge processes for maximal energy recovery during primary treatment, given further optimization of sludge separation.

Throughout the reactor experiments, a clear trend was observed toward a decreasing fraction of COD oxidized to CO_2 with decreasing SRT. The SRT is known to influence the extent of substrate mineralization even at the very low SRT that are typically employed in high-rate systems (Faust et al., 2014b; Jimenez et al., 2015). Further experimental and model-based research on the effect of varying SRT on the HiCS system should help determine optimal values of SRT in terms of energy recovery.

3.3 Sludge production

The observed sludge yield was compared for all reactors. As expected, the sludge yield tended to be higher for the high-rate reactors compared to the low-rate ones (**Figure 3.4**).

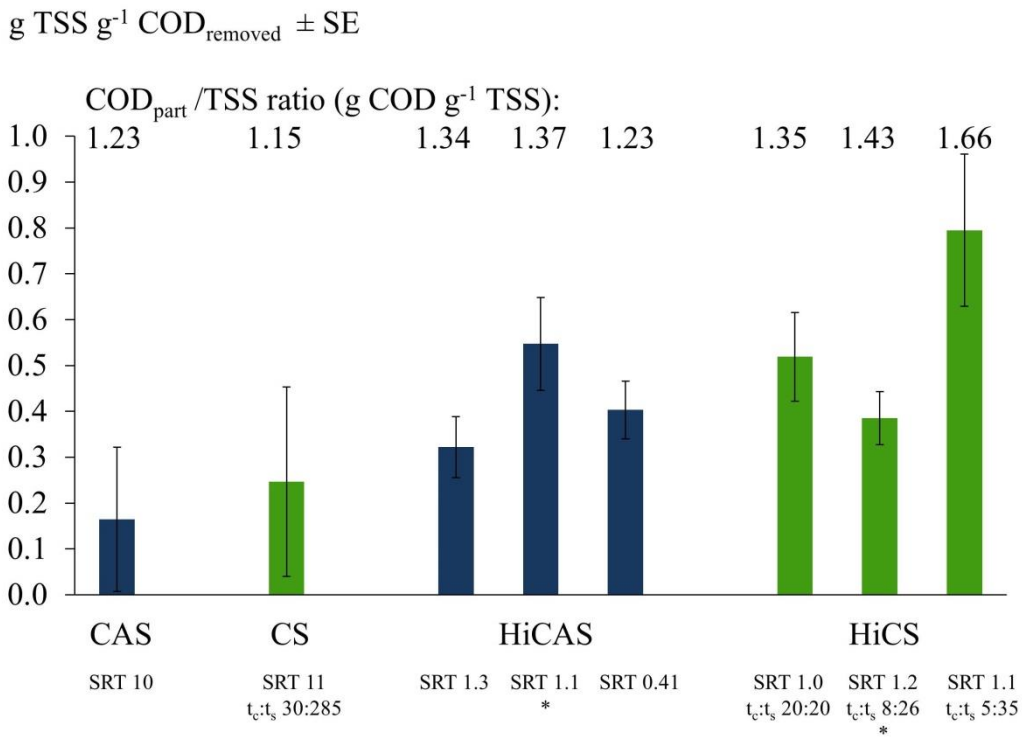


Figure 3.4: Observed sludge yield for the CAS, HiCAS, CS and HiCS reactors, alongside the COD_{part}/TSS ratios. Error bars show standard errors (SE). Reactors indicated with asterisk (*) were operated on diluted black water in CSTR mode; all other reactors were operated on synthetic wastewater in SBR mode.

Remarkably, the observed yield in the HiCS system at t_c:t_s 5:35 amounted to 0.71 g VSS g^{-1} COD, as recalculated based on the VSS/TSS ratio of 0.89 (**Table 3.3**). This was higher than the maximum yield of 0.4 to 0.5 g VSS g^{-1} COD for aerobic heterotrophic metabolism, as reported in literature (Sykes,

1975), and suggests the involvement of processes of sludge production other than aerobic cell growth in this reactor, substrate storage, or adsorption onto the existing sludge flocs, assuming that these do not require oxygen. However, due to high variability throughout the reactor runs, differences in yield were found not to be statistically significant.

3.4 Energy recovery as methane

The specific methane yield was determined for the reactors treating synthetic wastewater (**Table 3.4**) . The highest conversion of sludge to methane was obtained in the HiCS reactor at t_c:t_s 5:35 and the HiCAS reactors at SRT 0.41 and 1.31 d. Recalculated to volumes of methane produced per gram of sludge, the specific methane yield for these reactors amounted to 402, 484 and 389 mL CH_4 g^{-1} $TS_{sludge\ fed}$, respectively. These values were higher compared to the low-rate reactors ($0.05 > p$), but did not significantly differ from one another. For comparison, literature reports methane yields between 230 and 460 mL CH4 g^{-1} VS_{fed} for high-rate sludge (Ge et al., 2013; Nansubuga et al., 2015; Trzcinski et al., 2016a; Trzcinski et al., 2016b) and between 70 and 256 mL CH4 g^{-1} VS_{fed} for low-rate sludge (Bolzonella et al., 2005; Trzcinski et al., 2016a). By combining the COD removal efficiency, the sludge yield and the anaerobic methane yield, the overall recovery of $COD_{influent}$ as COD_{CH4} could be derived.

Table 3.4: Summary of COD removal, sludge yield, specific methane yield and overall energy recovery of the reactors treating synthetic wastewater. N.a.: not applicable. Standard errors are shown behind values or as error bars, where applicable. Significance groups for pairwise comparisons are shown next to the bars; if two bars share the same letter, they do not differ significantly ($p > 0.05$).

Reactor	SRT	t_c:t_s	COD removal	Sludge yield	Specific methane yield		Energy recovery
				g TSS	g COD_{CH4}		g COD_{CH4}
	d		%	g^{-1} $COD_{removed}$	g^{-1} $TS_{sludge\ fed}$	Significance	g^{-1} COD_{infl}
CAS	10	n.a.	74	0.16 ± 0.16	0.55 ± 0.04	b	0.07 ± 0.06
CS	12	30:285	46	0.25 ± 0.21	0.54 ± 0.06	b	0.06 ± 0.05
HiCAS	1.31	n.a.	55	0.32 ± 0.07	1.10 ± 0.13	ac	0.19 ± 0.05
	0.41	n.a.	48	0.40 ± 0.06	1.36 ± 0.04	a	0.27 ± 0.04
HiCS	1	20:20	40	0.52 ± 0.10	0.72 ± 0.07	bc	0.15 ± 0.03
	1.1	5:35	40	0.80 ± 0.17	1.13 ± 0.08	a	0.36 ± 0.08

Whereas the HiCAS reactors performed better in terms of COD removal percentage and specific methane yield, it was found that the HiCS reactor at t_c:t_s 5:35 had the highest overall COD recovery percentage, with 34% of the incoming COD recovered as methane. This was 33% higher than the energy performance of the second-best reactor; the HiCAS system at SRT 0.41 d. Comparison with values reported in literature shows that the COD recovery percentage of the HiCS system at t_c:t_s 5:35 was comparable to the 35% that was obtained in a high-rate membrane bioreactor (Akanyeti et al., 2010) and considerably higher than the typical values of around 25% in CAS systems (Cornel et al., 2011; Verstraete et al., 2009). The difference in energy recovery potential between the two HiCS reactors showed that contact and stabilization times have a major influence on the energy recovery

of the HiCS system, and that these parameters should, together with sludge separation and SRT, be further optimized. Due to the variability in the observed yields, however, the differences in energy recovery potential between the reactors was not significant. This indicates that the performance of the HiCS process is at least equivalent to existing high-rate systems but, given further optimization (see Chapters 4 and 6), potentially better.

3.5 Process kinetics of the HiCS system

Respirometric experiments were performed to improve understanding of the process kinetics of the HiCS system, and provide perspectives for further optimization. **Figure 3.5** presents the evolution of the $SOUR_{exo}$, i.e., a measure of the rate of substrate oxidation to CO_2, with the COD concentrations during one SBR cycle of the HiCS reactors treating synthetic wastewater.

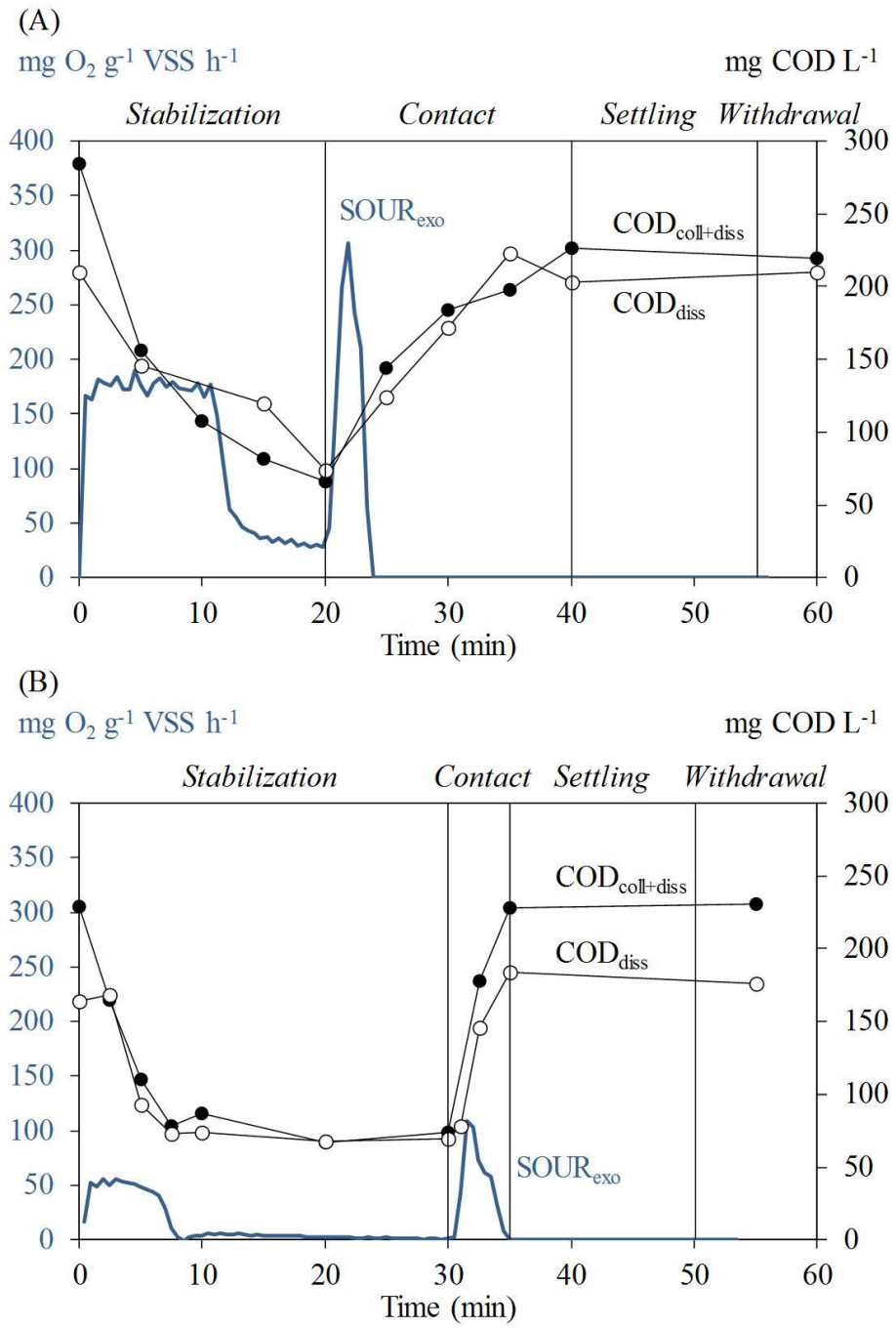


Figure 3.5: Kinetic characterization of the different phases of the in the HiCS reactors at (A) t_c:t_s 20:20 and (B) t_c:t_s 5.35. Exogenous SOUR is represented on the left axis; COD profiles on the right axis.

During stabilization, $SOUR_{exo}$ levels dropped drastically after approximately eight minutes in the HiCS reactor at t_c:t_s 5:35 and twelve minutes at t_c:t_s 20:20, marking the depletion of reserves of rapidly biodegradable COD. Only the sludge at t_c:t_s 5:35 was able to reach a near-endogenous state during stabilization, which means that a more pronounced substrate gradient was present in this reactor, and that selection pressures toward sorption and storage must have been considerably higher than

at t_c:t_s 20:20. In both reactors, DO levels dropped to nearly zero after five minutes of contact time because of a fast aerobic metabolism. Since the contact phase was not aerated, DO levels remained low (<0.07 mg L^{-1}) until aeration was switched on in the stabilization phase. The endogenous respiration rates, as measured prior to the respirometric batch tests, were 13.6 mg O_2 g^{-1} VSS h^{-1} for the reactor at t_c:t_s 20:20 and 9.06 mg O_2 g^{-1} VSS h^{-1} for the reactor at t_c:t_s 5:35. For each phase, removal percentages of dissolved and colloidal substrate are shown in **Table 3.5**.

Table 3.5: Changes in COD_{coll} and COD_{diss} concentrations (%) during the different phases of the SBR cycle of the HiCS reactors.

	SRT 1.0 d t_c:t_s 20:20			SRT 1.1 d t_c:t_s 5:35			
	COD_{coll}	**COD_{diss}**	**$COD_{coll+diss}$**	**COD_{coll}**	**COD_{diss}**	**$COD_{coll+diss}$**	
Stabilization	73	41	49	94	57	68	%
Contact	-188	14	7	-214	21	8	%
Settling and withdrawal	61	-3	3	-25	4	-1	%
Overall	63	47	48	-129	56	45	%

For both HiCS reactors, removal of COD_{coll} occurred mainly in the stabilization phase, and was negative in the contact phase, suggesting a net release of colloidal matter. However, absolute concentrations of COD_{coll} were low, and had only a minor effect on the overall removal of $COD_{coll+diss}$. COD_{diss} removal was more evenly distributed between the contact and stabilization phases, and was negligible during the settling and withdrawal phases. Over the entire cycle, the reactor at t_c:t_s 20:20 removed 202 mg $COD_{col+diss}$ and consumed 85 mg O_2, leading to an estimated growth yield of 0.58 g COD_{sludge} g^{-1} $COD_{coll+diss\ removed}$. The reactor at t_c:t_s 5:35 removed 190 mg $COD_{col+diss}$ and consumed 48 mg O_2, leading to an estimated growth yield of 0.75 g COD g^{-1} COD. Interestingly, the removal percentages in the five-minute contact phase of the reactor at t_c:t_s 5:35 were slightly higher than in the twenty-minute contact phase of the reactor at t_c:t_s 20:20 (**Table 3.5**). This suggests that removal during the contact phase is governed by physicochemical mechanisms such as sorption and flocculation. Indeed, it has been demonstrated that flocculation of activated sludge is essentially 99% completed within 10 minutes (Wahlberg et al., 1994). Zhao et al. (2000) found that flocculation and adsorption are responsible for 38% of the total COD removal in a laboratory-scale HiCS system, while the remaining 62% was mainly due to gravitational settling. When designing a HiCS system, a contact phase of only a few minutes may therefore be sufficient to reach a considerable removal of COD.

Conversely, stabilization times should be long enough to ensure sufficient degradation of slowly biodegradable COD, such as hydrolysable solids, polymers and storage products, and thus select for micro-organisms with a high storage and sorption capacity. This is in accordance to the findings of Huang and Li (2000), who found that a minimum stabilization period of 30 minutes is required to

ensure a sufficient biosorptive capacity of the sludge, and may explain the lower performance of the HiCS reactor at t_c:t_s 20:20, in which the stabilization period lasted only 20 minutes. No strong substrate gradient was reached in this reactor, because even though the SOUR declined after approximately twelve minutes of stabilization, presumably because of depletion of rapidly biodegradable COD, the reactor never reached a state of endogenous respiration.

Finally, not only the absolute retention times but also the t_c:t_s ratio likely influences overall removal efficiencies. In the low-rate CS system, this ratio determines the relative contribution of the different physical and biological removal processes in each phase (Gujer & Jenkins, 1975). Although the contact phase in the HiCS reactor at t_c:t_s 5:35 lasted only five minutes, COD_{diss} removal percentages were higher compared to the reactor at t_c:t_s 20:20, which suggests that an effective selection for micro-organisms capable of rapid sorption and storage is responsible for the superior energy recovery performance of the HiCS process at t_c:t_s 5:35.

4 Conclusions

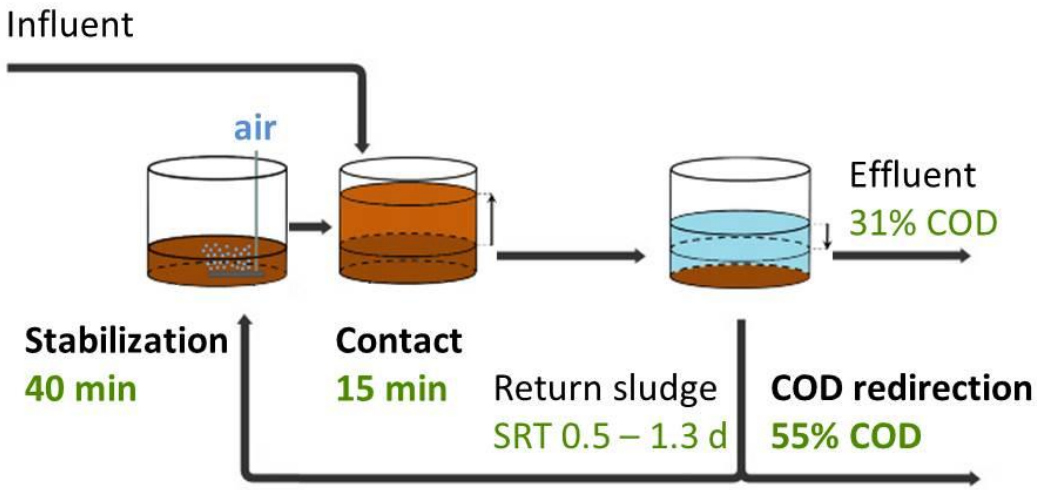
These experiments demonstrated that the HiCS process combines the advantages of the high-rate activated sludge process (i.e., high sludge yields and low percentages of substrate oxidation) with the advantages of the contact stabilization process (i.e., a feast-famine regime to select for rapid sorption and storage of substrates and achieve efficient removal of organic matter at very short contact times). Given further optimization of solid/liquid separation, SRT and t_c:t_s ratio, the HiCS process may outperform other high-rate activated sludge processes in terms of overall energy recovery, and can be considered a promising primary treatment process to obtain energy self-sufficient wastewater treatment.

5 Acknowledgements

F.A.M. and S.E.V. were supported as doctoral candidate (Aspirant) and as postdoctoral fellow, respectively, from the Research Foundation Flanders (FWO-Vlaanderen). We thank Giacomo Bellandi and Tinne De Boeck for the assistance with the respirometric experiments, Dries Seuntjens for the provision of sludge inocula, Jo De Vrieze for the assistance with the BMP experiments and the inspiring scientific discussions, and José Carvajal-Arroyo and Lars Angenent for their feedback on the manuscript.

CHAPTER 4:

OPTIMIZATION OF THE HIGH-RATE CONTACT STABILIZATION (HICS) SYSTEM



Cover figure: Performance of an optimized HiCS system.

This chapter has been redrafted after:

MEERBURG, F. A., BOON, N., VAN WINCKEL, T., PAUWELS, K. T. G. & VLAEMINCK, S. E. (2016). Live fast, die young: Optimizing retention times in high-rate contact stabilization for maximal recovery of organics from wastewater. *Environmental Science & Technology* **50**(17): 9781-9790.

1 Introduction

Conventional HRAS processes (i.e., HRAS processes with a conventional process configuration) struggle with the trade-off between minimizing respiration losses by lowering the SRT and maximizing effluent quality and biosorption performance by raising the SRT (Jimenez et al., 2015; Jimenez et al., 2007). To redirect a maximal amount of incoming organics into the sludge stream, optimization efforts should therefore primarily focus on enhancing adsorption and intracellular storage of organic matter at short SRT (see Introduction). Substrate adsorption, which occurs at the level of the extracellular polymeric substances (EPS) of sludge flocs, may be more influenced by loosely-bound EPS (LB-EPS) than by tightly-bound EPS (TB-EPS) (Liu et al., 2010). Storage of organic carbon mainly occurs in the form of polyhydroxybutyrate (PHB) (van Loosdrecht et al., 1997).

It has been suggested that the HiCS process reaches higher COD removal efficiencies compared to conventional HRAS systems (Zhao et al., 2000). Previous experiments found that the energy recovery by the HiCS system depends on the SRT and the hydraulic retention times during the contact and stabilization phases, with a suggested minimum contact time of 5 min and a minimal stabilization time of 30 min (Huang & Li, 2000; Chapter 3). Given the novelty of the HiCS process, research on the process is scarce, and its status as an alternative to conventional HRAS processes has remained hypothetical. The HiCS process has not been optimized for maximal organics recovery, nor is it known to what extent different biological pathways contribute to the overall removal of organics. In this study, the recovery of COD by means of adsorption and storage was investigated in the HiCS system at different combinations of SRT (0.24, 0.5, 1.3 and 2.8 d), contact time (8 and 15 min) and stabilization time (15 and 40 min) to identify an optimal operational strategy for recovering wastewater organics. Determination of the LB-EPS, TB-EPS and PHB content of the biomass was performed to estimate the relative contribution of adsorption and storage toward the overall removal of organic matter.

2 Materials and Methods

2.1 Reactor operation

Eight HiCS reactors were operated as sequencing batch reactors (SBR) with a working volume of 3 L. Synthetic wastewater was prepared according to Aiyuk and Verstraete (2004) with a complex organic fraction composed of acetate, peptone, starch, milk powder, dried yeast and soy oil, at a target concentration of 800 mg COD per liter (Aiyuk & Verstraete, 2004). At this COD concentration, the influent can be considered representative for high-strength domestic, or for industrial wastewater(Metcalf & Eddy, 2003). The influent was prepared in batches that were kept at 15°C and used within 2 days. All experiments were performed at a controlled temperature of 15°C. The reactors were inoculated with a 1:1 mixture of sludges from the high-rate and low-rate stages of the wastewater treatment plant of Nieuwveer (Breda, NL). Samples were taken near-daily after an

acclimation period of 3 to 6 days to reach a steady-state performance over several cycles of SRT. An orthogonal experimental design was implemented to optimize the SRT, contact time (t_c) and stabilization time (t_s) (**Table 4.1**). Wasting of sludge was performed during the contact phase. The waste flow rate was adjusted manually to maintain the desired SRT. The SBR cycle times were controlled by a microcontroller (Arduino, Turin, IT). All reactors had a contact phase of differing lengths, with a phase for filling and reacting and a phase for wasting, a settle phase of 40 min, a withdraw phase of 5 min, and a stabilization phase of differing lengths. After stabilization, an idle phase was implemented to synchronize the cycles of parallel reactors. **Table 4.1** lists the operational conditions of each reactor.

All experiments were performed in a temperature-controlled room at 15°C (standard deviation 1°C) to mimic average wastewater temperatures of Western Europe. Peristaltic pumps and pressurized air were used for pumping and aeration, respectively. Stirring was achieved with a magnetic stir bar at a 200-250 rpm rotational speed. Measurements of the dissolved oxygen (DO) concentration and pH in the reactors were made near-daily. Manual corrections of the air flow were made to ensure that DO levels were above 1.5 mg L^{-1} at the end of the stabilization phase, in order to remain well above the oxygen half-saturation coefficient of 0.2 mg L^{-1} for heterotrophic growth (Henze et al., 2000) and minimize possible effects of low DO concentrations on the production of EPS (Morgan et al., 1990) and PHB (Martins et al., 2003a). The pH was between 7.2 and 8.7. It should be noted that stabilization, contact and settling processes in SBR systems occur sequentially. Therefore, the SLR and the removal rates of SBR reactors are inherently lower compared to continuous systems, where these processes occur in parallel, and care should be taken when directly comparing rates between SBR and continuous systems.

Table 4.1: Overview of reactor operation, sludge characteristics and COD concentrations of the influent and effluent. SLR: sludge-specific loading rate, VER: volume exchange ratio. Standard errors are shown behind values, where applicable. (*) The bCOD/COD ratio was 0.99.

Reactor	**SRT_{tot}**	**SRT_{aer}**	**t_c:t_s**	**Steady-state operation**	**Influent flow rate**	**SLR**	**Stabilization volume**	**VER**	**Contactor VSS**	**VSS/TSS ratio**	**Influent COD**	**Effluent COD**
	d	d	min:min	d	L d^{-1}	g bCOD g^{-1} VSS d^{-1}	L	%	mg L^{-1}	%	mg COD L^{-1}	mg COD L^{-1}
A	**0.24 ± 0.01**	0.09 ± 0.004	15:40	21	16.0	8.1 ± 1.5	1.83	39	856 ± 97	95	919 ± 86	448 ± 40
B	**0.46 ± 0.03**	0.18 ± 0.01	15:40	26	21.4	4.7 ± 0.6	1.83	39	1160 ± 106	91	660 ± 31	249 ± 15
C	**1.31 ± 0.08**	0.50 ± 0.03	15:40	26	20.9	1.7 ± 0.1	1.83	39	2790 ± 130	92	668 ± 30	234 ± 22
D	**2.82 ± 0.38**	1.07 ± 0.15	15:40	18	21.0	1.5 ± 0.1	1.47	51	3920 ± 189	89	843 ± 52	322 ± 10
E	0.53 ± 0.02	0.22 ± 0.01	**8:40**	25	22.3	3.9 ± 0.2	1.53	49	1650 ± 95	93	834 ± 24	387 ± 27
F	0.50 ± 0.01	0.08 ± 0.002	**15:15**	25	22.3	4.8 ± 0.1	1.53	49	1300 ± 35	95	834 ± 20	464 ± 18
G	0.88 ± 0.03	0.37 ± 0.01	**8:40**	25	20.9	2.4 ± 0.1	1.62	46	2470 ± 100	92	844 ± 21	381 ± 16
H	0.85 ± 0.05	0.13 ± 0.01	**15:15**	25	20.9	3.2 ± 0.1	1.62	46	1850 ± 74	95	835 ± 12	504 ± 15

2.2 Coagulation and settling control experiments

Physicochemical coagulation and settling experiments of the synthetic wastewater were performed as controls to assess the performance of a primary settling treatment and a CEPT treatment under identical flow rates and reaction times as in the HiCS reactors B and C. For the primary settling experiment, influent was stirred at a 200-250 rpm rotational speed in a 3 L batch reactor for 15 min, and left to settle for 40 min. Prior to the CEPT experiment, the coagulants $Al_2(SO_4)_3$ and $FeCl_3$ were tested for their optimal concentration in a series of batch tests with a dosage between 0 and 300 mg L^{-1} for $Al_2(SO_4)_3$ and between 0 and 700 mg L^{-1} for $FeCl_3$. After 15 min of stirring and 40 min of settling, the transmission at 610 nm was measured spectrophotometrically. A dosage of 122 mg L^{-1} $Al_2(SO_4)_3$ resulted in the clearest supernatant, and was used to perform the CEPT experiment. Influent was stirred in a 3 L batch reactor with addition of this optimal coagulant dose, stirred for 15 min and left to settle for 40 min. The physicochemical experiments were performed in triplicate.

2.3 Extraction of EPS and PHB

A heat extraction protocol was modified from Li and Yang (2007) and Morgan et al. (1990) to extract the LB-EPS and TB-EPS fractions from the sludge. Sludge samples for EPS measurement were taken at the end of the contact phase. Sludge samples were immediately centrifuged at 4000*g* for 5 min and resuspended in 10 mL of Ringer's solution (9 g L^{-1} NaCl, 0.42 g L^{-1} KCl, 0.48 g L^{-1} $CaCl_2$ and 0.20 g L^{-1} $NaHCO_3$, adjusted to pH 7.0) diluted to 25% with distilled water and preheated to 60°C to ensure that the suspensions reached an immediate temperature of 50°C. After vortexing for 1 min, the samples were centrifuged at 4000*g* for 10 min to isolate the LB-EPS fraction in the supernatant. The pellets were resuspended in 10 mL diluted Ringer's solution, and heated at 60°C for 30 min. After centrifugation at 4000*g* for 15 min, the TB-EPS fraction was recovered from the supernatant. The EPS fractions and pellets were stored at -20°C until further processing. PHB extractions were performed on the sludge pellets after EPS extraction. The PHB extraction protocol was modified from Karr et al. (1983). Pellets were transferred to glass containers by resuspension in diluted Ringer's solution and centrifugation at 2000*g* for 30 min. Pellets were then dissolved in 5 mL of 98% H_2SO_4, and heated at 100°C for 20 min while vortexing every 5 min. After cooling to room temperature, the solutions were diluted 15 times and filtered through a 0.2 µm syringe filter (Chromafil PTFE, Macherey-Nagel, DE).

2.4 Analytical procedures

COD was measured using Nanocolor test kits (Macherey-Nagel, DE), according to the manufacturer's instructions. Five-day biochemical oxygen demand (BOD_5), total and volatile suspended solids (TSS and VSS) were determined according to standard methods (Greenberg et al., 1992). bCOD was calculated as BOD_5 divided by 65%, and the obtained value of 0.99 g bCOD g^{-1} COD for the synthetic wastewater was used throughout the calculations. COD concentrations were fractionated into total (COD_{tot}), particulate (COD_{part}), colloidal (COD_{coll}) and dissolved (COD_{diss}) fractions by filtering over a 20-25 µm paper filter (type 41, Whatman, GB) to remove particles and a 0.2 µm syringe filter (Chromafil PTFE, Macherey-Nagel, DE) to remove colloids. Nitrite, nitrate and phosphate were determined on a 761 Compact Ion Chromatograph (Metrohm, CH) equipped with a conductivity

detector. For protein determination, EPS samples were alkalified to a final concentration of 1 M of NaOH, and analyzed according to the Lowry protein assay (Lowry et al., 1951). Bovine serum albumin (BSA) was used as a standard by preparing a calibration series in 1 M NaOH from a stock of 1 g L^{-1} BSA stored at -20°C. For carbohydrate determination, samples were analyzed according to the anthrone method (Gerhardt et al., 1994). Glucose was used as a standard by freshly preparing a calibration series in water. EPS proteins and carbohydrates were expressed in mg of BSA and glucose equivalents, respectively. Digested PHB was measured as crotonic acid in a Dionex Ultimate 3000 HPLC system equipped with a Phenomenex Rezex ROA 8% column with a 2.5 mM H_2SO_4 solution as eluent and a flow rate of 0.6 mL min^{-1}. Crotonic acid was measured at a retention time of 30 min with an absorbance peak at 210 nm. Commercial PHBV (Goodfellow, GB) was used as a standard by preparing a calibration series in 98% H_2SO_4 and heating at 70°C for 2 min. Dilution, filtering and analysis was done parallel with the samples

2.5 Calculations and statistical comparisons

Removal of organic matter was addressed in terms of COD, to account for biodegradable and non-biodegradable organic matter. The daily observed sludge yield and daily COD balance over the reactors were calculated as described in Chapter 3. The SRT was calculated as $SRT = X_{SBR}V_{SBR} \times (X_eQ_e + X_wQ_w)^{-1}$, where X, V and Q are biomass concentration, volume and flow rate, respectively, and subscripts SBR, e and w denote the entire SBR reactor at the end of the contact phase, the effluent and the waste fraction, respectively. Unless reported otherwise, all reported values are averages of near-daily calculations, and dispersion is indicated by standard error. Statistical analyses were performed with the software R 3.0.2 (R Core Team, 2013). The Shapiro-Wilk test was used to test the normality of the data residuals, and the Levene's test to test the homogeneity of the variances. If the null hypothesis of normality was rejected, the Kruskal-Wallis rank sum test was used with the Mann-Whitney *U* test for post-hoc comparisons. In case of normally distributed data residuals, analysis of variance (ANOVA) was used if variances were homogenous, with the post-hoc student's *t*-test with pooled variance. The Welch's adaptations of the ANOVA and *t*-test were used if variances were not homogenous. Post-hoc tests were only performed if the null hypothesis of equal means was rejected, and *p*-values were always adjusted with the Benjamini-Hochberg correction. Differences were considered significant at a *p*-value below 0.05. Correlation analysis was performed with the averages of operational variables and performance indicators for each of the eight reactors (n = 8) to calculate the Spearman rank correlation coefficient ρ. Correlations were considered meaningful if ρ was significantly different from zero and its absolute value larger than 0.7.

3 Results

3.1 Removal efficiencies and sludge production

With an increasing SRT from 0.24 to 2.8 d (reactors A to D), the total COD removal efficiency increased significantly from 52% at SRT 0.24 d to 65% at SRT 1.3 d, after which it decreased slightly to

60% at SRT 2.8 (not significant) (**Figure 4.1**). At an SRT of 0.5 d, decreasing t_c from 15 (reactor B) to 8 min (reactor E) did not result in a lower COD_{tot} removal, while a decrease of t_s from 40 to 15 min (reactor F) significantly lowered the removal efficiency to 44%. The same trend was observed at higher SRT (reactors C, G and H), but since the effective SRT of these reactors did not equal their designed SRT of 1 d, quantitative comparison is preliminary. Correlation analysis revealed that only the t_s was significantly correlated to the removal efficiency of COD_{tot} and COD_{diss}, with a positive correlation coefficient (**Table 4.3**, see below). It should be noted that correlation analysis may only detect linear or monotonic associations between variables, and no non-linear associations, as may be the case between the SRT and the removal efficiencies for COD_{part}, COD_{coll} and COD_{diss}, which showed an optimum at an intermediate SRT.

Removal of COD_{part} contributed most to the total COD removal, with removal percentages between 80 and 93% which did not differ significantly among the reactors. Regarding the influence of SRT and t_c:t_s ratio, the removal percentages of dissolved COD followed the same trends as for COD_{tot}; the highest COD_{diss} removal efficiency was observed at an SRT of 1.3 d (34%, reactor C) and lowering the t_s from 40 to 15 min significantly lowered the COD_{diss} removal efficiency to a mere 5%. The lowest and most variable removal efficiencies were observed for colloidal COD, which was even negative in reactor A (**Figure 4.1**).

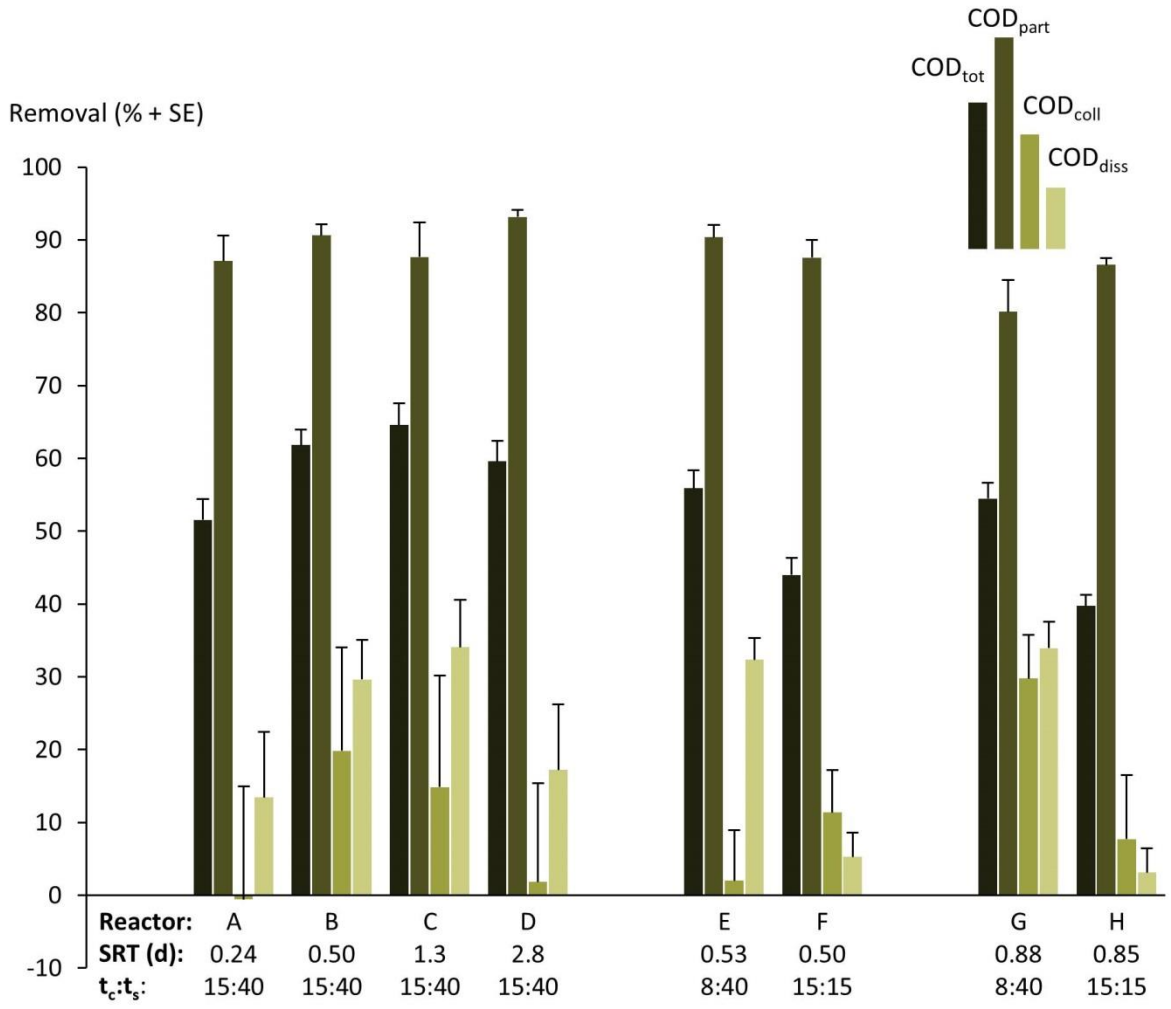


Figure 4.1: COD removal efficiencies for the different reactors. Error bars show standard errors (SE).

The observed sludge yield showed a decreasing trend with increasing SRT (**Figure 4.2**). The lowest yield was observed at an SRT of 2.8 d (reactor D), which was significantly lower than in the reactors with a shorter SRT, where the observed yield approximated the theoretical maximum of 1 g COD_{sludge} g^{-1} $COD_{removed}$. Changes in t_c and t_s had no significant effect on the observed sludge yield. This was confirmed by correlation analysis (**Table 4.3**, see below), where the yield was significantly correlated to the SRT (ρ = -0.88) but not to the t_c or t_s.

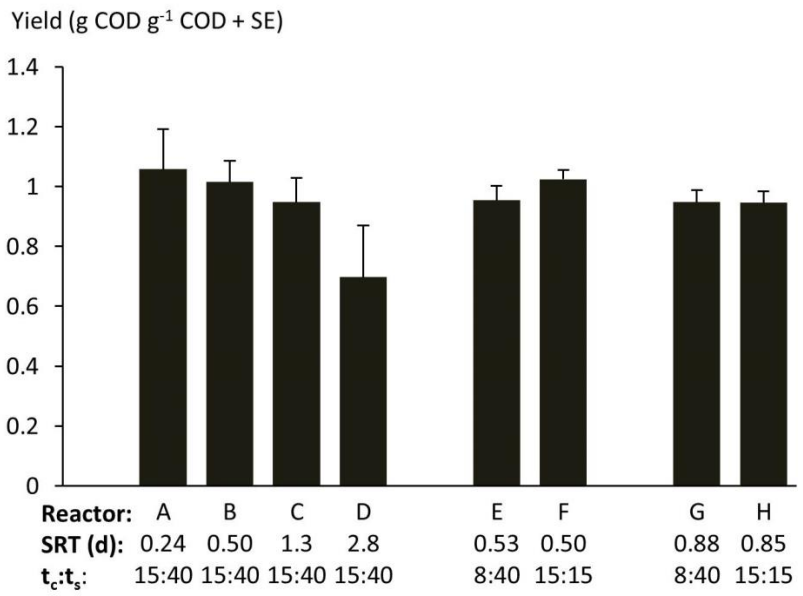


Figure 4.2: Observed sludge yield for the HiCS reactors. Error bars show standard errors (SE).

The influent contained, on average, 0.04±0.08 mg L^{-1} NO_2^--N, 2.4±0.8 mg L^{-1} NO_3^--N and 4.1±2.9 mg L^{-1} PO_4^{3-}-P. Whereas no nitrite and nitrate were added to the synthetic wastewater, these values were consistent with the nitrite and nitrate concentrations in the tap water used for the synthetic wastewater. Removal efficiencies of combined nitrite and nitrate ranged between 59% and 92%, presumably due to denitrification, with the highest removal in reactors C and G. Removal of orthophosphate ranged between 20% and 63%, with the highest removal in reactor B.

It should be noted that there were variations in mixed-liquor VSS concentrations among reactors as a result of the different SRTs, and this resulted in a range of different SLRs (**Table 4.1**). Therefore, care should be taken when directly comparing performances between reactor A, which had a very high SLR, and reactors C and D, whose SLR was in the lower range of values obtained. For the same reason, any effects of SRT on reactor performance described in this study may, in part, also be due to the coupled differences in SLR. Moreover, a lowering of the t_s caused a decrease in SRT_{aer}. While in most reactors, the ratio of aerobic to total SRT remained relatively constant between 0.37 and 0.42, this ratio dropped to 0.16 in the reactors where the t_s was lowered to 15 min (reactors F and H). Any difference in reactor performance due to a decrease of the t_s may therefore also be explained by the concomitant decrease in the SRT_{aer}.

3.2 Recovery of organic matter

An overall COD balance was made over each reactor to monitor the fractions of COD that entered and exited the reactor systems via waste sludge, effluent and losses to oxidation, as presented in **Figure 4.3**.

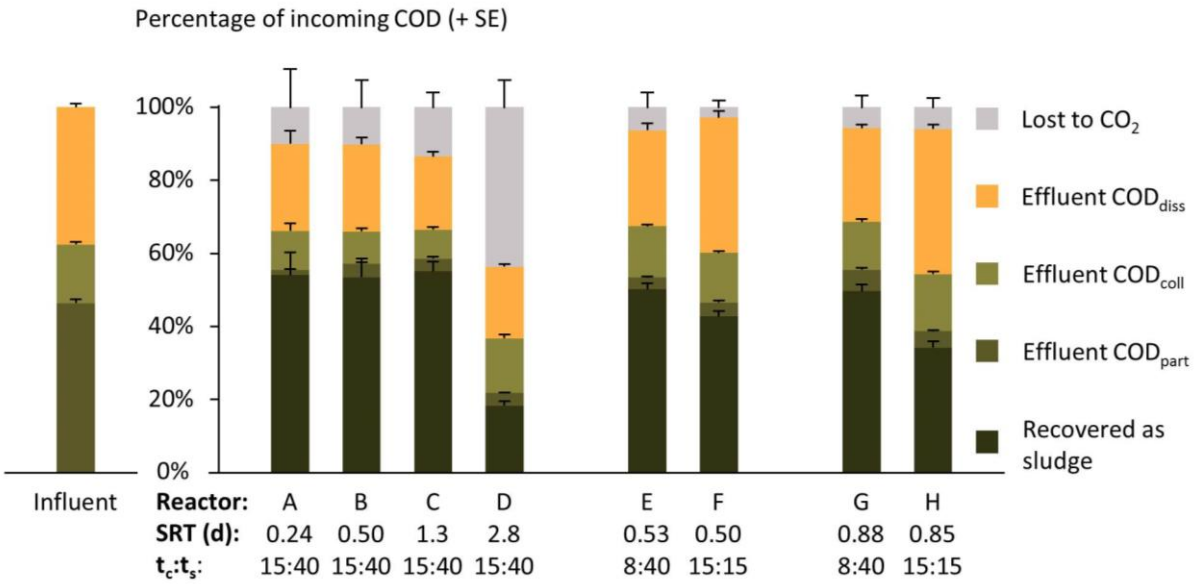


Figure 4.3: Average balance of COD fractions leaving each reactor, relative to incoming COD. Error bars show standard errors (SE).

The best performance was observed at the three lowest SRTs (reactors A, B and C), with average COD recovery percentages of 54, 53 and 55% and oxidation percentages of 10, 10 and 14%, respectively, which did not differ significantly. Only at the longest SRT of 2.8 d (reactor D), a clear effect of the SRT on reactor performance could be observed, with a significantly lower recovery of 18% and an oxidation of 44%. While changes in t_c and t_s did not significantly change the fraction of COD oxidized, a decrease in t_c from 15 to 8 min (in reactors E and G) resulted in a significantly higher fraction of colloidal COD in the effluent and a decrease in t_s from 40 to 15 min (reactors F and H) resulted in a significant reduction in the fraction of recovered sludge and significant increases in the fractions of colloidal and dissolved COD in the effluent. Correlation analysis did not show strong associations between the COD recovery percentage and operational variables (**Table 4.3**, see below), but the fraction of COD_{diss} leaving the reactors was negatively correlated to the t_s.

Physicochemical coagulation and settling experiments were performed to assess the COD removal efficiency and organic matter recovery of a primary settling treatment and a CEPT treatment under identical conditions of influent composition, flow rates and reaction times as in the HiCS reactors B and C. The results are presented in **Table 4.2** and compared to the HiCS reactors B and C, which had the best performance of all reactors in terms of COD removal efficiency and organic matter recovery. Primary settling of the influent resulted in a poor removal efficiency and organic matter recovery, whereas coagulation with $Al_2(SO_4)_3$ followed by settling resulted in an organic matter recovery equal to that in the optimal HiCS reactors. However, the HiCS reactors performed significantly better in terms of COD removal efficiency since, besides recovery, an additional part of the incoming COD was oxidized.

Table 4.2: Comparison of COD removal efficiency and organic matter recovery of the primary settling and CEPT experiments, and the best-performing HiCS reactors (B and C).

Reactor	**SRT**	**t_c:t_s**	**COD removal efficiency**				**Organic matter recovery**
			Particulate	Colloidal	Dissolved	Total	
	d		%	%	%	%	%
Settling			81.6 ± 1.2	20.7 ± 5.5	-1.0 ± 0.8	30.1 ± 1.0	30.6 ± 1.0
Coagulation + settling			79.2 ± 2.8	60.0 ± 2.0	22.4 ± 0.6	49.8 ± 1.4	50.5 ± 1.3
HiCS B	0.46	15:40	90.6 ± 1.6	19.8 ± 14.2	29.7 ± 5.4	61.8 ± 2.1	53.2 ± 6.9
HiCS C	1.31	15:40	87.6 ± 4.8	14.9 ± 15.3	34.1 ± 6.6	64.6 ± 3.0	55.1 ± 3.4

3.3 Extracellular polymeric substances and poly-β-hydroxybutyrate

The concentrations of proteins and carbohydrates in the tightly bound EPS fractions were always two to eight times higher than in the loosely bound EPS fractions (**Figure 4.4**).

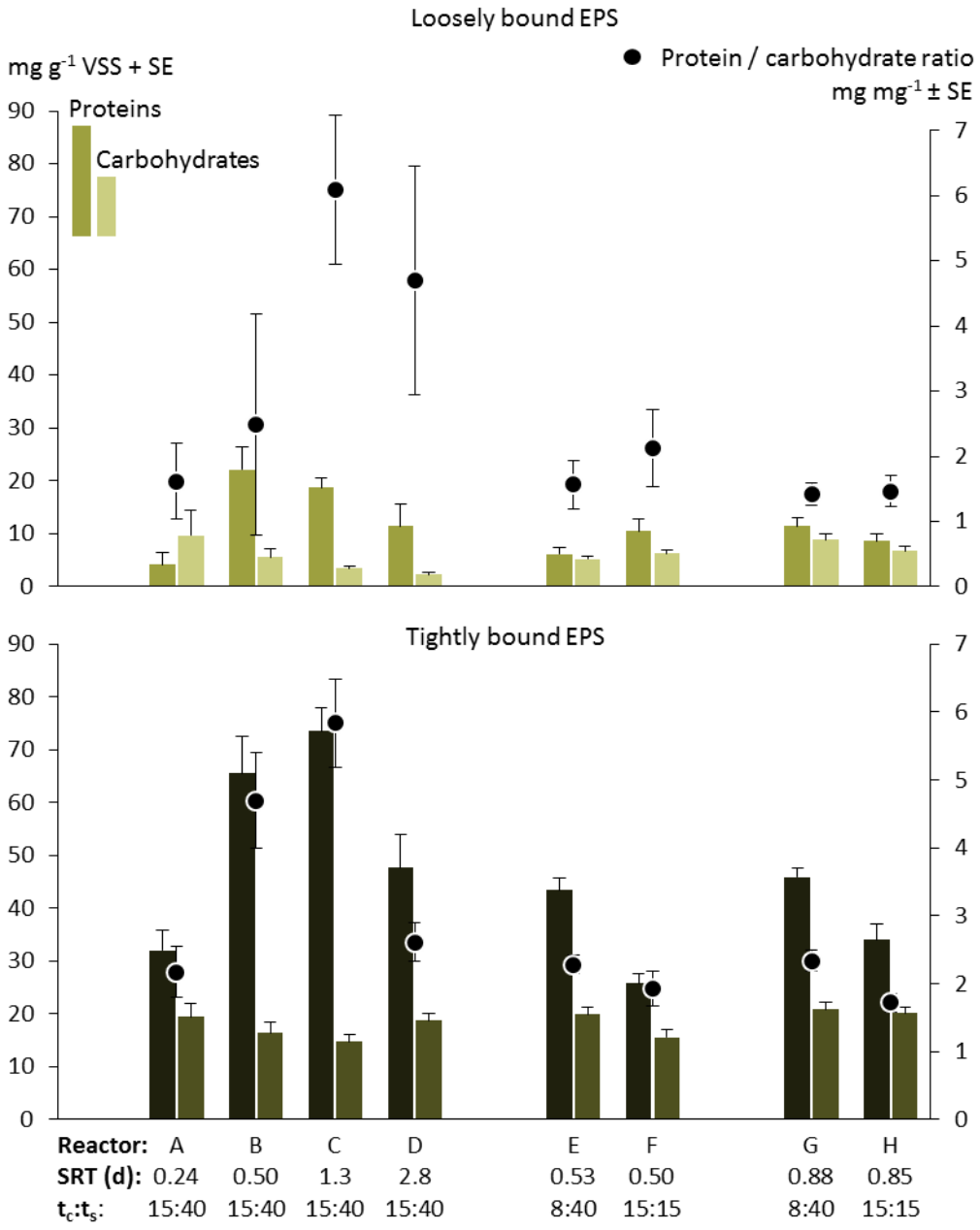


Figure 4.4: Specific protein and carbohydrate concentrations of the loosely (top) and tightly bound (bottom) EPS fractions. Error bars show standard errors (SE).

In the LB-EPS fraction, carbohydrate concentrations showed a gradual decline with increasing SRT, to a minimum of 2.3 g g^{-1} VSS at an SRT of 2.8 d (reactor D). In the TB-EPS fraction, the carbohydrate concentrations remained stable around a level of 18 g g^{-1} VSS, and none of the reactors showed significant differences. Protein concentrations were more variable, and were the main factor determining changes in the protein-to-carbohydrate (P/C) ratio. The P/C ratio significantly increased with increasing SRT to a maximum of 6 mg mg^{-1} at an SRT of 1.3 d (reactor C) in both the LB-EPS and TB-EPS fractions, after which the ratio declined again at an SRT of 2.8 d (reactor D). At an SRT of 0.5

d, a decrease of the t_c to 8 min (reactor E) and t_s to 15 min (reactor F) had no significant influence on the protein-to-carbohydrate ratio in the LB-EPS fraction, but caused a strong decrease in the TB-EPS fraction, compared with a t_c:t_s ratio of 15:40 min (reactor B). Correlation analysis showed that the EPS composition did not monotonously correlate to operational variables (**Table 4.3**). Correlations with COD removal efficiency were generally negative for the carbohydrate fractions and positive for the protein fractions of the EPS. The strongest correlations were between the protein content in the TB-EPS fraction and the COD_{tot} (ρ = 0.90) and COD_{diss} removal efficiency (ρ = 0.74), between the protein content in the LB-EPS fraction and the COD_{coll} removal efficiency (ρ = 0.79), and between the carbohydrate fraction in the LB-EPS fraction and the COD_{part} removal efficiency (ρ = -0.83).

The intracellular PHB concentration at the end of the contact phase showed a strong influence of the SRT, with a significant increase from a concentration of 2.6 to 21 mg g^{-1} VSS with increasing SRT up to 1.3 d (reactor C), followed by a strong decline by 41% at an SRT of 2.8 d (**Figure 4.5**). A change in the t_c did not result in differences in PHB concentration, but a decrease in t_s from 40 min (reactor B) to 15 min (reactor F) caused a significant PHB decrease by 60% at an SRT of 0.5 d. Correlation analysis showed no monotonous correlation between the PHB content and operational variables (**Table 4.3**). The PHB content was, however, positively correlated to the removal efficiencies of total (ρ = 0.95), particular (ρ = 0.71) and dissolved (ρ = 0.74) COD. Furthermore, the PHB content was negatively correlated to the carbohydrate fraction of LB-EPS (ρ = -0.83) and positively to the protein fraction of the TB-EPS (ρ = 0.86). Measurements with a ten-minute interval within each reactor cycle showed that PHB concentrations never changed considerably from feast to famine phase.

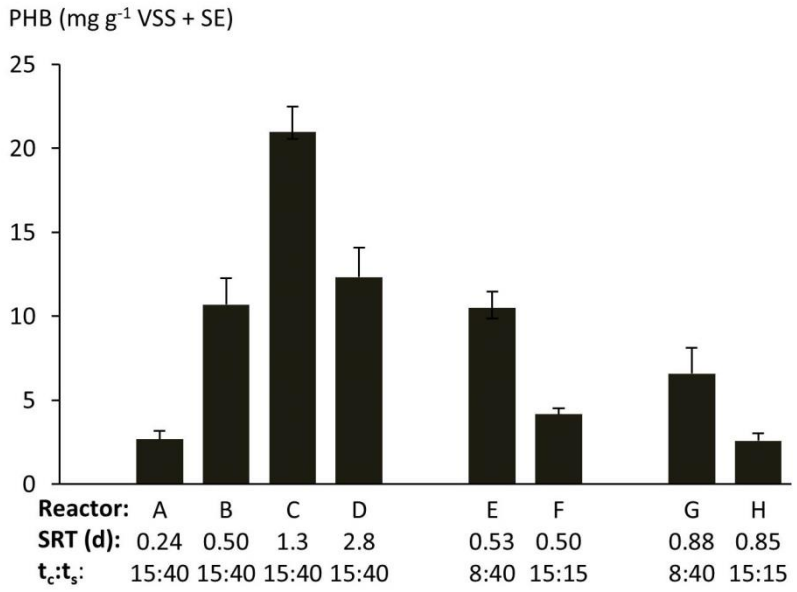


Figure 4.5: Intracellular PHB concentrations for the HiCS reactors. Error bars show standard errors (SE).

Table 4.3: Spearman rank correlation coefficients for operational variables and performance indicators of the eight HiCS reactors. Colors indicate when ρ is significantly different from zero and its absolute value larger than 0.7 (light) or 0.9 (dark), where negative correlations are indicated in red and positive correlations in green.

		SRT_{tot}	SRT_{aer}	t_c	t_s	B_X	Removal efficiency				Yield	COD balance					EPS			
							COD_{tot}	COD_{part}	COD_{col}	COD_{diss}		Recovered	COD_{part}	COD_{col}	COD_{diss}	Oxidized	C-LB	C-TB	P-LB	P-TB
SRT_{aer}		0.36	-																	
t_c		-0.13	-0.25	-																
t_s		0.13	0.63	-0.33	-															
B_X		-0.98	-0.38	0.13	-0.25	-														
Removal efficiency	COD_{tot}	0.33	0.17	0.00	0.76	-0.45	-													
	COD_{part}	0.14	0.07	0.25	0.38	-0.21	0.67	-												
	COD_{col}	0.14	-0.50	-0.25	0.00	-0.19	0.26	-0.24	-											
	COD_{diss}	0.38	0.24	-0.50	0.76	-0.45	0.79	0.17	0.48	-										
Yield		-0.88	-0.33	0.13	0.00	0.90	-0.12	-0.07	-0.10	-0.12	-									
COD balance	Recovered	-0.48	0.10	-0.63	0.25	0.52	-0.14	-0.33	-0.07	0.29	0.62	-								
	COD_{part}	0.38	-0.14	-0.38	-0.50	-0.31	-0.50	-0.43	0.38	-0.21	-0.55	-0.21	-							
	COD_{col}	0.26	0.14	-0.13	-0.50	-0.19	-0.60	-0.05	-0.43	-0.60	-0.52	-0.26	0.62	-						
	COD_{diss}	-0.19	-0.26	-0.25	-0.76	0.31	-0.90	-0.52	-0.17	-0.64	-0.02	0.19	0.64	0.74	-					
	Oxidized	0.31	0.57	0.38	0.63	-0.40	0.67	0.62	-0.43	0.29	-0.21	-0.33	-0.67	-0.24	-0.76	-				
EPS	C-LB	-0.60	-0.05	-0.13	-0.25	0.62	-0.69	-0.83	0.05	-0.36	0.43	0.43	0.17	-0.05	0.45	-0.55	-			
	C-TB	0.02	0.38	-0.63	0.00	-0.05	-0.50	-0.55	-0.05	-0.12	-0.33	0.24	0.55	0.52	0.55	-0.36	0.52	-		
	P-LB	0.36	-0.29	0.13	0.25	-0.45	0.67	0.29	0.79	0.52	-0.26	-0.48	0.07	-0.50	-0.62	0.14	-0.40	-0.40	-	
	P-TB	0.52	0.14	0.00	0.63	-0.67	0.90	0.48	0.43	0.74	-0.43	-0.38	-0.24	-0.45	-0.81	0.60	-0.62	-0.29	0.79	-
PHB		0.52	0.17	0.00	0.63	-0.60	0.95	0.71	0.24	0.74	-0.26	-0.24	-0.33	-0.43	-0.81	0.62	-0.83	-0.55	0.67	0.86

4 Discussion

4.1 Reaching sufficient effluent quality

The HiCS system is designed to be the primary stage of a two-stage activated sludge system, because the operational strategy to achieve maximal redirection of organic carbon in HRAS systems (i.e., lowering the SRT and raising the SLR) typically result in a deterioration of overall COD and nutrient removal efficiencies (Haider et al., 2003; Jimenez et al., 2015; Jimenez et al., 2007). In this study, the COD removal efficiency increased with SRT, but declined again at the longest SRT of 2.8 d, primarily because of lower removal of colloidal and dissolved COD. This may be due to the combined effects of improved biosorption with increasing SRT, and a higher incidence of hydrolysis or release of soluble microbial products (SMP). Indeed, experiments with high-rate membrane bioreactors (MBR) at short SRT showed an increase in SMP with increasing SRT between 0.5 and 2 d (Teksoy Başaran et al., 2012). Within the range of short SRTs tested here, an optimum was found between 0.5 and 1.3 d.

To allow organic matter removal in activated sludge by the relatively rapid processes of sorption and bioflocculation, studies have shown that a minimal contact time of 5 or 10 minutes was required (Bunch & Griffin, 1987; Wahlberg et al., 1994). In this study, the fraction of recovered COD did not differ significantly when the t_c was lowered from 15 to 8 min, but a higher fraction of colloidal COD left the system through the effluent. Possibly, the bioflocculation process was not complete after 8 min. A longer contact time of 15 min is therefore recommendable to obtain a more stable operation of the HiCS system and effluent quality.

A strong decrease in COD removal was observed when the t_s was lowered from 40 to 15 min. This indicates that a stabilization period of 15 min is not sufficient for the sludge to deplete its adsorbed or stored COD and regenerate, and is in accordance with Huang and Li (2000), who found that stabilization times should be at least 30 min. Tsang et al. (2006) found that the relative ratio of feast-to-famine retention times determines the strength of the selection pressure toward bioflocculation. According to the kinetic selection theory (Daigger & Grady, 1982), both adsorption and storage should therefore be favored when stabilization times are longer. An optimal t_s for the HiCS system is therefore more likely to be found above 35 min (Meerburg et al., 2015) or 40 min, as observed in this study.

Comparison between physicochemical coagulation and settling tests demonstrated that the HiCS system at these optimal SRTs achieves a higher overall COD removal efficiency compared to CEPT, especially in the particulate and dissolved COD fractions. The CEPT control experiment removed 22% COD_{diss}, and is in concurrence with previous studies who found that alum-based CEPT can remove some COD_{diss} by adsorption onto the formed $Al(OH)_3$ gel (Haydar & Aziz, 2009). However, the CEPT experiment was not optimized for factors such as shear force and reaction times, and a fully optimized CEPT system may achieve a better COD recovery than what was obtained in this study.

The best effluent quality was obtained by reactor C, with an average COD concentration of 234 ± 22 mg L^{-1}. Whereas absolute effluent COD concentrations will differ according to the specific influent

strength and composition, this value is far above the effluent standards of many urbanized regions, such as the standard of 125 mg L^{-1} COD set forth by the European Union (Directive 91/271/EEC). Moreover, the potential for biological nitrogen and phosphorus removal is limited in high-rate systems, due to the short SRT (Henze et al., 2008; Mulkerrins et al., 2004). Therefore, high-rate systems typically require further polishing of organics and nutrients from the effluent during secondary treatment (Verstraete & Vlaeminck, 2011). To attain energy-neutral wastewater treatment, optimization efforts should combine the optimization of organics recovery in HRAS systems with the development and implementation of energy-efficient technologies for downstream nutrient removal. For example, anammox-based deammonification requires 60% less energy for nitrogen removal compared to conventional nitrification/denitrification (Mulder, 2003), and much work is being performed to improve the feasibility of anammox-based nitrogen removal under mainstream conditions (Gao et al., 2014). For efficient deammonification, a low bCOD/N ratio is desirable (De Clippeleir et al., 2013b), presumably below 1.4 g bCOD g^{-1} N (Jenni et al., 2014; Zhang et al., 2015b). Since raw domestic wastewater typically has a ratio above 9 g bCOD g^{-1} N (Metcalf & Eddy, 2003), optimization of the HiCS system should primarily focus on redirecting organic carbon.

4.2 Improving the adsorption and storage response

The removal of COD_{tot} peaked to more than 60% in the reactors with an SRT of 0.5 d and 1.3 d. Because the extent of oxidation only varied around 10%, these reactors effectively removed organic matter by means of conservational mechanisms, such as adsorption and storage. The COD removal efficiencies were correlated with higher concentrations of proteins and lower concentrations of carbohydrates in the EPS. The positive correlation between the EPS protein concentrations and COD removal supports the hypothesis that the diverse positive, negative and neutral moieties of proteins play a role in the adsorption of organics (Sheng et al., 2010; Späth et al., 1998), and concurs with the experimental results of Xie et al. (2010), who suggested that EPS proteins play a more important role in bioflocculation than carbohydrates. The protein-to-carbohydrate ratio peaked at an intermediate SRT of 1.3 d. This is similar to what was observed by Faust et al. (2014b), but contradicts the results of Ng and Hermanowicz (2005), who found a decrease in EPS protein-to-carbohydrate ratio in a high-rate MBR with SRTs increasing from 0.25 to 5 d, or the results of Jimenez et al. (2015), who found that the total EPS content of sludge in a conventional HRAS installation increased as the SRT increased from 0.3 to 1.5 d, and then leveled off as the SRT further increased to 5 d. Faust et al. (2014b) operated a series of high-rate MBRs with SRTs between 0.125 and 5 d, and observed a peak in EPS protein concentrations at an SRT of 1 d. They hypothesized that this peak was caused by the combined effect of increasing EPS formation at very low SRTs between 0.125 and 0.5 d, due to increasing microbial activity, and increasing EPS degradation at SRTs above 1 d, due to increasing endogenous decay of biomass-associated polymers, and due to increasing metabolic diversity, where more species capable of EPS degradation may be present. Possibly, this hypothesis may not only explain the peak in EPS concentrations at an SRT of 1.3 d in this study, but also the similar peak of intracellular PHB concentrations at the same SRT (Figure 4.5).

The nearly constant level of TB-EPS carbohydrates in all reactors may be consistent with the results of Yang and Li (2009), who found that the TB-EPS content of activated sludge reactors remained relatively constant under changing conditions of carbon source in the synthetic wastewater, SRT and loading rate. The TB-EPS proteins varied among reactors and showed strong positive correlations with the COD_{tot} and COD_{diss} removal efficiencies (**Table 4.3**). This suggests that the formation of TB-EPS proteins may be associated with the metabolic conversion of soluble substrates. Conversely, the LB-EPS protein content showed a strong positive correlation with the COD_{coll} removal efficiency. This corresponds to the observation that the bioflocculation performance of sludge is mainly associated with the LB-EPS fraction, as has been shown in literature (Liu et al., 2010; Yang & Li, 2009). Further work should be performed to determine the contribution of different EPS fractions towards the removal of COD_{part}, COD_{coll} and COD_{diss}. Whereas the complex synthetic wastewater in current study was selected to reflect realistic amounts of COD_{part} and COD_{diss}, organic and ammonia-nitrogen, as found in raw domestic sewage (Aiyuk & Verstraete, 2004), differences in influent composition and strength may result in changes in LB- and TB-EPS production when the HiCS system is operated with real wastewater of medium to low strength. It should also be noted that EPS measurement is sensitive to the specific extraction method (Sheng et al., 2010), and the existence of several often-used methods may prevent direct comparison among reported measurements in literature.

The settling characteristics of the sludge were addressed indirectly through monitoring the effluent quality and organics recovery performance of the reactors. In all reactors, the constant settling time of 40 min and maximum travel distance of 7.5 cm subjected the sludge to an equivalent upflow velocity of 0.11 m h^{-1}, which is far below the range of 1.3 – 3 m h^{-1} typically employed in primary treatment stages with sludge return (Metcalf & Eddy, 2003), and was deemed sufficiently low to minimize effects of differences in sludge settleability on reactor performance in this study. However, additional optimization of the settleability and bioflocculation properties could further improve the solids/liquid separation and organics recovery in the HiCS system, as evidenced by the presence of particulate and colloidal COD in the effluent of all reactors.

The COD removal efficiency strongly correlated with intracellular PHB concentrations. The PHB production did change with variations in the SRT and stabilization time, but no temporal changes were observed within the feast-famine cycles of the reactors (data not shown). Feast-famine cycling is known to select for sludge with a strong storage response, even at very short SRTs below 1 d (Majone et al., 1999). The highest level of PHB observed in this study was 21 mg g^{-1} VSS (reactor C), which was considerably lower than the 40 mg g^{-1} VSS that may be achieved in low-rate wastewater treatment systems optimized for PHA production (Morgan-Sagastume et al., 2015). Considering the COD/weight ratio of 1.67 g COD g^{-1} PHB and COD/VSS ratio of 1.58 g COD g^{-1} VSS in reactor C, the production of PHB only accounted for 2.3% of the recovered COD. This suggests that production of storage polymers was not the predominant route of COD removal in these experiments, and that biosorption, biomass growth and settling were responsible for most of the COD removal. Beun et al. (2002) reviewed a number of studies that subjected activated sludge to feast-famine conditions with acetate as carbon source, and demonstrated that the fraction of consumed acetate going to PHB

production remains constant at a range of SRTs >2 d, but that PHB production is less predictable at lower SRT. In a pilot HRAS study with real wastewater at an SRT of 0.5 d, Haider (2002) demonstrated that PHB only accounted for 2% of the influent organics. Possibly, the similarly low percentage of storage in current study was a result of the short contact and stabilization times, which may have been too short to facilitate the reaction chain of biological uptake, conversion and polymerization during feast and subsequent degradation and mineralization of the polymer during famine to selectively favor micro-organisms with a storage-dependent growth cycle. Instead, the low levels of PHB were likely a result of a response to shock loading (Ni et al., 2014). Nonetheless, the fast feast-famine cycles of the HiCS system may be sufficient to selectively favor the relatively fast processes of EPS-mediated bioflocculation and intracellular accumulation of organic matter without chemical modification (Chudoba et al., 1982; Majone et al., 1999).

In these experiments, an SRT of 1.3 d was found to result in optimal COD removal, COD recovery, EPS production and internal storage. Because of the use of synthetic influent, the reactors were not continuously seeded with sewage micro-organisms that could constitute a substantial fraction of the sludge community and contribute in the conversion and recovery of COD, as is the case in full-scale HRAS processes treating sewage (Gonzalez-Martinez et al., 2016). Whereas seeding of influent micro-organisms lowers the minimal SRT required to retain sludge in the system (see Chapter 1, Section 5.2), it is postulated that the presence and extent influent seeding does not have a direct influence on the SRT at which the HiCS system achieves optimal adsorption and storage. This is because a selection pressure for adsorption and storage can only take effect on micro-organisms capable of growing and reproducing within the system, irrespective of influent seeding. The COD removal and recovery performance, on the other hand, may be influenced by influent seeding, because of substrate competition between the autochthonous community, adapted for optimal sorption and storage, and the unadapted seeding community. Therefore, the optimization results obtained in this study may not be suitable to quantitatively predict the performance of a HICS system with a different influent strength and composition.

4.3 Reaching energy neutrality

The overall redirection of COD is determined by the product of COD removal and observed sludge yield. Up to an SRT of 1.3 d, the yield approached 1 g COD g^{-1} COD, which is well above the maximum yield of 0.4 – 0.7 g COD g^{-1} COD for heterotrophic growth (Metcalf & Eddy, 2003). Yields higher than the heterotrophic growth yield may indicate that COD is removed by processes other than cell growth, such as storage (Dircks et al., 1999), and it is not uncommon that growth yields reported for HRAS processes approach the upper limit of 1 g COD g^{-1} COD (Jimenez et al., 2015). In this work, the sludge yield was dependent on the SRT, which is a well-described phenomenon (Metcalf & Eddy, 2003), but not by the t_c and t_s. At extremely short SRTs below 0.5 d, it is difficult to maintain a viable sludge mass in an activated sludge reactor. Therefore, of all operational conditions tested, the optimal conditions of the HiCS system in terms of carbon redirection and recovery are a t_c of 15 min, a t_s of 40 min and an SRT between 0.5 and 1.3 d. The carbon recovery percentages of 53% to 55% are higher than for conventional HRAS systems with an SRT between 0.5 and 1 d, which achieve a

recovery between 27% and 41% of incoming COD to sludge (Jimenez et al., 2015), although direct comparison of these values is difficult because of differences in influent composition and operational conditions.

An estimation of the potential energy recovery by means of AD can be made, considering that the methane yield during AD of HiCS sludge with an SRT of 1 d was reported to be 0.73 g $COD_{methane}$ g^{-1} $COD_{sludge\text{-}fed}$ (Meerburg et al., 2015, Chapter 3). Thus, digestion of recovered sludge from the HiCS reactors in this study would result in an energy recovery of 39±5% at an SRT of 0.5 d and 40±3% at an SRT of 1.3 d, expressed as percentage of influent COD that is finally recovered as $COD_{methane}$. With a conversion factor of 203 g COD kWh^{-1} - a conservative estimation (Heidrich et al., 2011), - a methane energy content of 3.86 $\times 10^{-3}$ kWh g^{-1} COD, and an electricity conversion efficiency of 0.35 kWh_{el} $hWh_{methane}^{-1}$ in a combined heat and power (CHP) installation (U.S. EPA, 2007), this would allow an annual recovery of 27.1 and 27.9 kWh of electricity per population equivalent (PE_{COD110}), respectively. Müller and Kobel (2004) determined that typical large CAS plants in Germany equipped with a CHP unit can produce 16.4 kWh PE_{BOD60}^{-1} y^{-1} from combustion of biogas. This is equivalent to about 13.3 kWh PE_{COD110}^{-1} y^{-1} for sewage with standard characteristics (Metcalf & Eddy, 2003). Thus, a full-scale HiCS system could potentially achieve an energy recovery of more than twice as high as that of a CAS system. It should be noted that the methane yield from HRAS sludges may differ depending on the type of sludge, wastewater and anaerobic digestion conditions, and values between 44% and 73% $COD_{methane}$ $COD_{sludge\text{-}fed}^{-1}$ have been reported (Ge et al., 2013; Meerburg et al., 2015; Nansubuga et al., 2015; Trzcinski et al., 2016b). Digestibility experiments of HiCS sludge should therefore be performed to estimate of the energy recovery potential of the HiCS system under various conditions.

However promising, the results of this work should be validated at various conditions to reflect full-scale wastewater treatment, where there may be variations in wastewater flow rate, strength, composition, temperature and other characteristics. In case of sewage treatment from a combined sewer system, additional resilience is required of the activated sludge system to deal with periodic dilution of sewage and increases in flow rates due to rainfall. Fluctuations in wastewater flow rate create changes in the reactor HRT, which may negatively impact the COD recovery performance, for example by limiting the extent of substrate adsorption in the reactor and causing particle washout from the settler when the HRT becomes too short, or by increasing the extent of COD oxidation in the reactor and causing substrate hydrolysis in the settler when the HRT becomes too long. A continuous HiCS system may provide better protection against load variations and shocks than a conventional HRAS system, because at any time, a major fraction of the sludge resides in the stabilization reactor and is not in contact with the influent (Sarioglu et al., 2003). Compared to the stability of controlled laboratory experiments, the fluctuating conditions of full-scale systems may result in a higher diversity of the activated sludge community (Saikaly & Oerther, 2004), which may in turn favor functional redundancy and resilience of the system (Saikaly & Oerther, 2011). However, the presence of fluctuating conditions may warrant the use of safety margins when designing a full-scale HiCS system, and the choice of SRT, t_c, t_s and other operational parameters should be made

both in function of maximizing organics recovery as well as maintaining a sludge community dense and resilient enough to withstand perturbation.

To move toward a mature technology that economically competes with CEPT, primary settling, and conventional HRAS processes, the HiCS process should be further optimized for COD recovery. Different primary treatment technologies should be compared in terms of COD recovery performance, energy balance, and operational and investment costs when coupled to secondary treatment, to determine the advantages and disadvantages of each technology. For example, CEPT may have a lower investment cost compared to a HiCS system, but higher operational costs due to chemical dosage, and a lower sludge digestibility due to the presence of coagulants in the sludge (Cabirol et al., 2003).

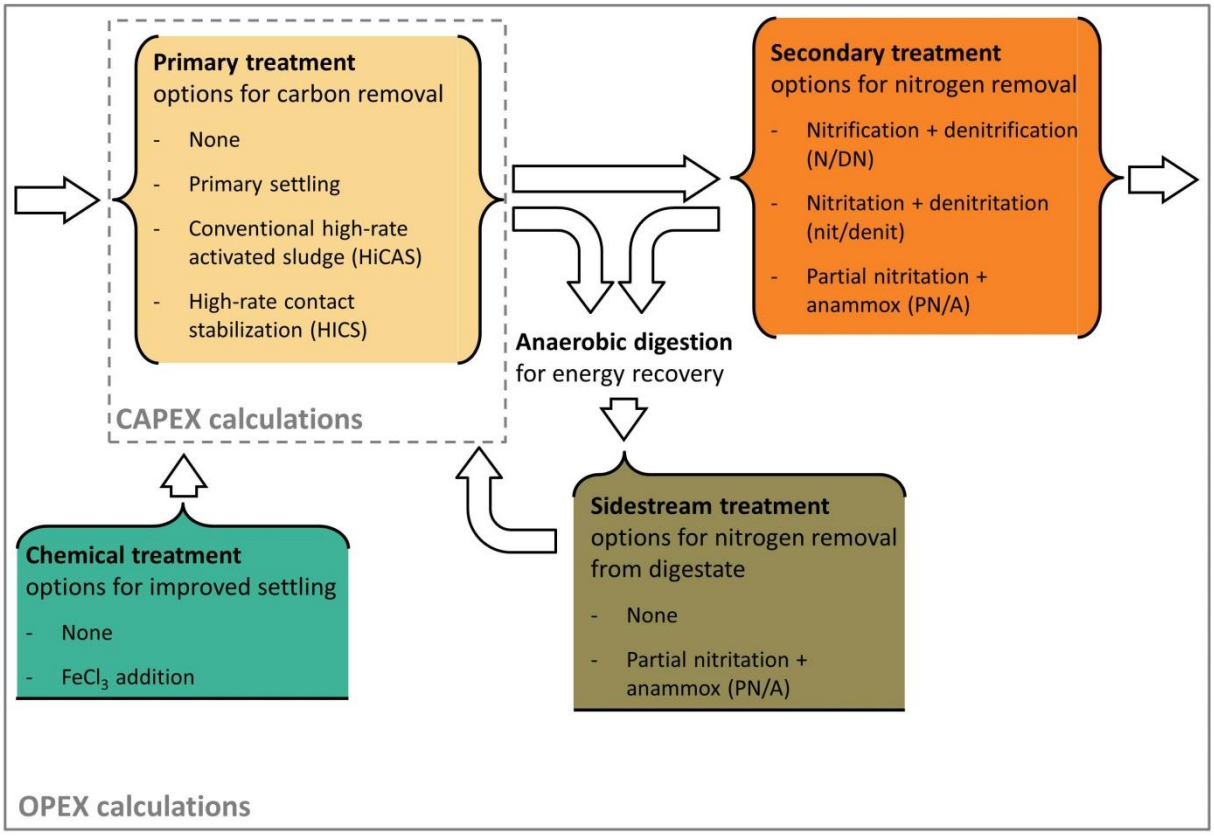
In conclusion, this study showed that high-rate contact stabilization as a primary wastewater treatment technology was able to recover up to 55% of incoming organic matter as sludge, which could be used for electrical energy recovery. The major share of organic matter removal was attributed to conservational mechanisms such as bioflocculation, biomass growth and settling, as opposed to oxidation. To reach maximal energy recovery, the best results for this study were obtained for the HiCS process at a sludge age between 0.5 and 1.3 d, a contact time of 15 min and a stabilization time of 40 min. Future research should focus on further improving colloidal and dissolved COD recovery efficiencies, and validating the performance of the HiCS system under various full-scale wastewater treatment conditions, in comparison with other primary treatment technologies.

5 Acknowledgements

F.A.M and S.E.V were supported, respectively, as doctoral candidate (Aspirant) and postdoctoral fellow by the Research Foundation Flanders (FWO-Vlaanderen). N.B. was supported by the Inter-University Attraction Pole (IUAP) ‘μ-manager’ funded by the Belgian Science Policy Office (BELSPO, P7/25). We thank Bert Bundervoet for the provision of sludge inocula, Dries Seuntjens and Samnang Nop for their aid in developing the Arduino controller, Veerle De Smedt, Bavo Vanneste and Greet Van De Velde for practical work and Jo De Vrieze for feedback on the manuscript.

CHAPTER 5:

WHICH PRIMARY TREATMENT TECHNOLOGY SHOULD WE USE? SCENARIO ANALYSIS FOR DIFFERENT AB-SYSTEMS AND ECONOMIC EVALUATION



Cover figure: Overview of scenario options and abbreviations. CAPEX: capital expenditure. OPEX: operational expenditure.

This chapter has been redrafted after:

MEERBURG, F. A., SEUNTJENS, D., BOON, N. & VLAEMINCK, S. E. (in preparation). Comparison of the energy and cost balances of full-scale municipal wastewater treatment with different technologies for carbon and nitrogen removal.

1 Introduction

The development of wastewater treatment technologies that allow recovery of resources, such as fresh water, energy and nutrients, is an important aspect of the transition towards a more sustainable water cycle. To maximize energy recovery and achieve a sufficient effluent quality, wastewater treatment can be performed as a two-stage system (AB-system, see Chapter 1). A modern AB-system may combine any physicochemical or biological technology for organics recovery during primary treatment with any low-rate treatment technology for nutrient removal during secondary treatment. The high-rate contact stabilization (HiCS) system is a promising technology to achieve organics recovery (see Chapters 3 and 4). Thus far, it has not been investigated to what extent the incorporation of the HiCS process into an AB-system will affect overall plant performance.

Several criteria can be used to evaluate whether a technology is suitable for 'sustainable' wastewater treatment, although some indicators of 'sustainability' may contradict each other. Energy neutrality is not the same as carbon neutrality. Some plants claim to be 'carbon neutral', simply by offsetting their energy consumption by on-site electricity generation (Suez Environment, 2014; U.S. EPA, 2014). However, it can be argued that, to be truly carbon neutral, any greenhouse gas emissions by the plant – be they CO_2, N_2O, CH_4 or other greenhouse gases expressed as CO_2-equivalents – need to be compensated for. Furthermore, energy-neutral or even carbon-neutral WWTPs are not necessarily sustainable, because residual organics, nutrients and other (micro)pollutants in the effluent may still have adverse environmental effects (Sweetapple et al., 2015). When criteria such as climate impact, contribution to biodiversity loss, and adverse effects on human health have to be weighed against each other, which is currently a subjective decision, it becomes even less clear which wastewater treatment approach can be considered most 'sustainable' (Schaubroeck et al., 2015).

The choice for a specific wastewater treatment technology ultimately depends on economics as the decisive criterion. If a sustainable treatment technology is to be adopted widely, it should offer an economic benefit over the alternatives. There is a need to understand the impact of adopting a specific technology for resource recovery on the overall WWTP performance, energy balance and cost balance. For example, plant-wide optimization should not only focus on maximizing organics recovery in the A-stage, but also on keeping the COD/N balance in the B-stage sufficiently high to accommodate nitrogen removal, depending on the specific technology. In this context, it should be noted that the economic viability of a sustainable technology may change when environmental impacts are included in the operational costs of wastewater treatment, e.g., by means of levies for greenhouse gas emissions.

The purpose of this study was to evaluate how using a HiCS system as primary treatment stage will affect the overall WWTP operation. First, reactor experiments were performed to assess whether incremental improvements in reactor performance can be obtained when an existing A-stage is converted from HiCAS to a HiCS configuration under constant operational conditions, and whether these incremental changes are repeatable / reversible. Second, an economic assessment was made

of different scenarios for an AB-installation, using a combination of technologies for organics and nitrogen removal. The A-stage consisted of (1) no treatment, (2) a primary settling system, (3) an A-stage with a high-rate conventional activated sludge (HiCAS) configuration, or (4) a high-rate contact stabilization (HiCS) system, each time with and without addition of chemical coagulants to improve floc formation. The B-stage consisted of an (1) N/DN, (2) nit/denit, or (3) PN/A system. Sidestream treatment consisted of anaerobic digestion of waste sludge, followed by dewatering with (1) no treatment of the liquid fraction prior to its return to the A-stage, or (2) N removal from the liquid fraction by PN/A. An economic evaluation was made, based on operational costs (OPEX) and capital costs (CAPEX) for expanding an existing stand-alone CAS installation to an AB-system.

2 Materials and methods

2.1 HiCAS and HiCS configuration experiments

An SBR reactor with a working volume of 3 L was operated first in HiCAS mode (i.e., the configuration of a conventional A-stage), then switched to HiCS mode, and reverted back to HiCAS. Synthetic wastewater was prepared according to Aiyuk and Verstraete (2004) with a COD concentration of 460 ± 80 mg L^{-1}. The synthetic wastewater was prepared in batches that were kept at 15°C and used within 4 days. The experiment was performed at a controlled temperature of 15°C. The inoculum consisted of a 1:1 mixture of A-sludge and B-sludge from the WWTP of Breda (the Netherlands). Samples were taken every two or three days after an acclimation period of 1.5 weeks for each switch in reactor operation mode. Sludge wasting was performed at the end of the contact phase and the waste flow rate was adjusted manually to control the SRT according to the equation $SRT = X_{SBR}V_{SBR} \times (X_eQ_e + X_wQ_w)^{-1}$, where X, V and Q are biomass concentration, volume and flow rate, respectively, and subscripts SBR, e and w denote the SBR reactor at the end of the contact phase, the effluent and the waste fraction, respectively. The aerobic SRT (SRT_{aer}) was calculated as the total SRT multiplied by the fraction of total cycle time that the reactor DO was above 0.5 mg L^{-1}. **Table 5.1** lists the operational details of the reactor experiments.

Table 5.1: Overview of reactor operational details. In case standard deviations are shown, n = 5 to 6. N.a: not available / applicable.

	HiCAS (1)	HiCS	HiCAS (2)	
Design parameters				
t_c:t_s	n.a.	15:40	n.a.	min:min
Reactor times:				
Stabilization	0	40	0	min
Contact (fill+react)	46	13	53	min
Contact (waste)	2	2	2	min
Settle	40	38	38	min
Withdraw	5	2	2	min
idle	2	0	0	min
Stabilization volume	n.a.	0.7	n.a.	L
Volume exchange ratio	77	77	77	%
Influent flow rate	34.8	34.8	34.8	L d^{-1}
Results				
Steady-state operation	14	19	7	d
SRT_{tot}	0.91 ± 0.21	0.92 ± 0.35	0.81 ± 0.07	d
SRT_{aer}	0.41 ± 0.15	0.28 ± 0.21	n.a.	d
VSS (end of contact phase)	2.4 ± 0.4	2.4 ± 0.4	1.8 ± 0.4	g L^{-1}
VSS/TSS ratio	0.92 ± 0.02	0.92 ± 0.02	0.90 ± 0.02	
SLR	2.7 ± 0.5	2.1 ± 0.2	2.6 ± 0.5	g bCOD g^{-1} VSS d^{-1}
Influent COD	540 ± 50	450 ± 60	390 ± 50	mg L^{-1}
Effluent COD	170 ± 70	230 ± 30	110 ± 30	mg L^{-1}
COD_{tot} removal	68 ± 16	47 ± 14	72 ± 7	%
COD_{part}	67 ± 21	56 ± 16	59 ± 11	%
COD_{coll}	57 ± 19	15 ± 39	60 ± 22	%
COD_{diss}	66 ± 18	32 ± 8	90 ± 2	%
N_{part} removal	56	33	n.a.	%
N_{diss} removal	5.4	-0.59	n.a.	%

Peristaltic pumps and pressurized air were used for pumping and aeration, respectively. Stirring was achieved with a magnetic stir bar at 800-1000 rpm. During HiCAS mode, the reactor was aerated in the contact phase, while during HiCS mode, aeration was performed in the stabilization phase only. In the aerated phases, the DO was controlled (SC200, Hach, U.S.A.) at a level between 2.0 and 2.5 mg L^{-1}.

The COD concentrations were measured using Nanocolor test kits (Macherey-Nagel, Germany). Fractionation between COD_{part}, COD_{col} and COD_{diss} was performed by filtering over a 1.5 µm paper filter (type 934-AH, Whatman, United Kingdom) to remove particles, and performing flocculation-filtration (0.45 µm) to remove colloids according to the protocol by Mamais et al. (1993). The TSS, VSS, NH_4^+ and KjN were determined according to standard methods (Greenberg et al., 1992). Fractionation of nitrogen into N_{part} and N_{diss} was done by filtering samples over a 0.45 µm syringe filter. Nitrite and nitrate were determined with a 761 Compact Ion Chromatograph (Metrohm, Switzerland), equipped with a conductivity detector. The COD balances were calculated with a

correction for imperfect separation of settleable and non-settleable organics, i.e., from the COD_{tot} that was measured in the waste fraction, the COD_{coll} and COD_{diss} were subtracted and added to the effluent fraction, taking into account differences in flow rate of the waste and effluent streams, so that the corrected waste fraction contained only COD_{part}. In other words, it was assumed that COD_{part} was settleable and COD_{coll} and COD_{diss} was non-settleable.

2.2 Coagulation experiments

For chemical coagulation, the optimal dosage ratio of $FeCl_3$ (expressed as mg $FeCl_3$ mg^{-1} $COD_{part+coll}$) was determined by adding known amounts of $FeCl_3$ to a series of jars containing 100 mL of influent, HiCAS or HiCS MLSS. Jars were stirred with a magnetic stirring bar for 10 seconds at 500 rpm, 2 minutes at 150 rpm, 3 minutes at 100 rpm and 15 minutes on 'shaking' mode at 100 beats per minute. The pH was not corrected. Samples were allowed to settle for 38 minutes - the same settling time as in the reactor experiments - and the optical density of the supernatant was measured at 610 nm to determine at which dosage of $FeCl_3$ the highest amounts of particulate and colloidal matter could settle. At this optimal dosage, the COD_{part}, COD_{coll} and COD_{diss} concentrations of the supernatant were measured to determine the percentage of COD redirection to the settled sludge fraction relative to the controls where no $FeCl_3$ was added. The COD balances for the continuous scenarios with primary settling, HiCAS and HiCS treatment with chemical coagulation were then calculated from the COD balances without coagulant addition, by subtracting the additional percentages of redirection of COD_{part}, COD_{coll} and COD_{diss}, as determined in the coagulation batch tests, from the effluent and adding it to the sludge fraction. For primary settling, the COD balance was assumed to consist of 22.5% redirection of COD_{part} to sludge (Siegrist et al., 2008).

2.3 Scenario analysis

Scenario calculations were performed in Microsoft Excel 2010. Because of circular dependence (e.g., COD and N in the reject water from the side stream were returned to the primary treatment, and caused an increase in primary sludge production, which in turn increased the flux going through the side stream), calculations were performed iteratively, until a maximum of 100 iterations was reached or the change of the cell values was < 0.1%.

2.3.1 Influent and effluent characteristics

All case study analyses were based on the raw influent and final effluent of the municipal AB-WWTP in Breda (The Netherlands) in the period of October 2013 to July 2014. Data for COD_{tot}, N_{tot}, TSS and flow rates were determined according to standard methods (Greenberg et al., 1992) obtained by personal communication with Waterschap Brabantse Delta (the Netherlands), who owns and operates the plant. To give a realistic approximation of daily energy usage and OPEX, average influent and effluent characteristics (from 24-hour composite samples, collected proportionally to the influent flow rate in order to correct for diurnal flow variations) were used for the calculations, for both wet and dry weather. To ensure that the plant remained functional during peak flow conditions, plant dimensions and CAPEX were calculated using the peak flow rate, which was calculated as the 95^{th} percentile of the flow data from the Breda WWTP. The COD and N concentrations of the raw

influent were used as such, while TSS and VSS concentrations were calculated from the TSS of the raw influent to reflect values after grit removal. For this purpose, it was assumed that the VSS/TSS ratios of the influent before and after grit removal were 0.67 and 0.73, respectively, and that 10% of TSS was removed as grit (Metcalf & Eddy, 2003). Population equivalents (PE) were calculated as $(COD_{in} + 4.57\ N_{in}) \times (150\ g\ TOD\ PE^{-1}\ d^{-1})^{-1}$. Additional fractionation of influent COD and N was based on standard values from literature, as summarized in **Table 5.2**.

Table 5.2: Characteristics of the influent and effluent, as used for the scenario analyses.

Variable	Value	Unit	Reference
Influent			
Population equivalents (PE_{TOD150})	375301		Calculated
Flow rate	80100	$m^3\ d^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
Peak flow rate (95th percentile)	486000	$m^3\ d^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
COD	485	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
N_{tot}	48	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
TSS (raw)	255	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
TSS (after grit removal)	230	$mg\ L^{-1}$	Metcalf & Eddy (2003)
VSS (after grit removal)	168	$mg\ L^{-1}$	Metcalf & Eddy (2003)
VSS/TSS (after grit removal)	0.73		Metcalf & Eddy (2003)
N_{part}/N_{tot} ratio	0.17		Henze et al. (2008)
$COD_{part+coll}/COD_{tot}$	0.6		Henze et al. (2008)
COD_{diss}/COD_{tot}	0.4		Henze et al. (2008)
$COD_{coll}/COD_{part+coll}$	0.11		Breda WWTP, 2012
$COD_{part+coll}$/TSS	1.27		Calculated
Effluent			
COD_{tot}	37	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
N_{tot} (max. concentration)	10	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014
TSS	7.2	$mg\ L^{-1}$	Breda WWTP, Oct 2013 - Jul 2014

2.3.2 Primary treatment

The influent of the primary treatment was composed of raw influent (after grit removal) and the return flow from the side stream. During primary settling, 22.5% of COD_{tot} and 10% of N were redirected to the waste stream (Siegrist et al., 2008). It was assumed that COD and N removal were only due to settling of the particulate fractions, not the colloidal or dissolved fractions. During conventional A-stage treatment (i.e., with the HiCAS configuration) and HiCS treatment, redirection of COD and N was based on the COD balances of the reactor experiments (relative to the influent COD_{tot}).

For the scenarios with chemical coagulation, COD balances were calculated from the COD balance of the primary settling, HiCAS and HiCS systems without chemical addition and the additional percentage of redirection of COD_{part}, COD_{coll} and COD_{diss} from the supernatant to the sludge due to chemical flocculation, as determined in the coagulation experiments. For primary settling, dosage of

the coagulant ($FeCl_3$) was based on the $COD_{part+coll}$ of the incoming streams (raw influent + side stream return flow). For the HiCAS and HiCS systems, coagulant dosage was based on the outgoing load of $COD_{part+coll}$ from the system (waste fraction and effluent). Removal of nitrogen was assumed to be the same as in the scenarios without chemical flocculation.

The production rate of primary sludge was estimated from the COD balances (expressed as % of incoming COD load redirected to the waste stream). Sludge COD_{part}/VSS ratios were used as determined in the HiCAS and HiCS experiments. The sludge COD_{part}/TSS and VSS/TSS ratios were calculated by taking into account the load of inorganic TSS coming in through the influent. It was assumed that the COD_{part}/VSS and VSS/TSS ratios of the sludge were equal to those in the primary effluent. The effect on these ratios of inorganic TSS coming in from the side stream was considered negligible. When applicable, the daily dosage of coagulants was added to the inorganic fraction of the waste TSS. The nitrogen content of the waste stream was estimated from the nitrogen removal efficiencies, assuming that no losses of nitrogen as N_2 occurred during primary treatment.

2.3.3 Secondary treatment

For secondary treatment, all scenarios were set to reach effluent concentrations as listed in **Table 5.2**, where effluent COD_{tot} and TSS were constant, and effluent N_{tot} could be lower than the maximum value, depending on the scenario. Influent nitrogen was assumed to be KjN hydrolyzed to NH_4^+-N, while effluent nitrogen was assumed to be NO_3^--N. Effluent TSS concentrations were considered non-biomass TSS, i.e., all produced biomass was assumed to leave the system through the waste stream. Removal of COD and N was approached as follows:

- Of all catabolic COD (i.e., COD_{in} – COD_{eff} – $COD_{sludge\ growth}$), 20% was always oxidized aerobically (Matějů et al., 1992). To close the COD balance, the calculation of catabolic COD did not take into account the fraction of heterotrophic biomass grown on methanol;
- Of all catabolic nitrogen (i.e., NH_4^+-N_{in} – $N_{sludge\ growth}$), 10% was always completely nitrified to NO_3^--N and left the system through the effluent;
- In the N/DN and nit/denit scenarios, the remaining catabolic N was removed entirely by nitrification/denitrification or nitritation/denitritation, respectively. This consumed the remaining COD partially or completely;
- In the PN/A scenarios, the remaining catabolic nitrogen was removed by partial nitritation/anammox, which resulted in a production of 0.111 g NO_3^--N g^{-1} NH_4^+-N removed, according to the stoichiometry of the PN/A process (Barnes & Bliss, 1983; Strous et al., 1998). This additional nitrate was considered to leave the system through the effluent;
- A hybrid nit/denit + PN/A scenario was created in those cases where there was insufficient COD to allow nit/denit. In this scenario, N was removed through nit/denit until depletion of the oxidizable COD, after which the remaining N was removed by PN/A;
- In case the NO_3^--N content of the effluent was too high, additional denitrification was performed until the maximum effluent concentration was reached;
- Any excess of COD was oxidized aerobically;

- Any shortage of COD during denitrification was supplemented by addition of an external carbon source (methanol). The dosage of methanol also accounted for the fraction of $COD_{methanol}$ used by the denitrifying heterotrophs for biomass growth.

Biomass production was estimated from the net amounts of catabolic COD or N removal and the observed biomass yields during each of the above steps. Observed yields were calculated from maximum yields according to the equation $Y_{obs} = Y_{max}$ $(1+f_d \times b_T \times SRT)$ $(1+b_T \times SRT)$. Values for Y_{max} were 0.63 g $COD_{biomass}$ g^{-1} COD_{rem} for aerobic heterotrophic growth and 0.54 g $COD_{biomass}$ g^{-1} COD_{rem} for anoxic heterotrophic growth (Henze et al., 2000), 0.21 g $COD_{biomass}$ g^{-1} $N_{removed}$ for nitritation (Blackburne et al., 2007a) as well as for nitratation (Blackburne et al., 2007b), assuming that NOB were primarily composed of *Nitrospira*, and 0.186 g $COD_{biomass}$ g^{-1} $N_{removed}$ for the PN/A process (combined growth of AOB and anammox bacteria) (Barnes & Bliss, 1983; Strous et al., 1998). The endogenous fraction f_d was assumed to be 0.2, and b_T was calculated as $b_{20} \times 1.04^{(T-20)}$, where b_{20} is 0.24 d^{-1} for heterotrophs, 0.06 d^{-1} for AOB and NOB and 0.005 d^{-1} for anammox (Van Haandel & van der Lubbe, 2007). Given the stoichiometric ratio of 39.2% anammox $COD_{biomass}$ to total $COD_{biomass}$ produced in the PN/A process (Barnes & Bliss, 1983; Strous et al., 1998), it was assumed that the combined decay rate for the PN/A sludge at 20°C was 0.038 d^{-1}. A value of 34.7 d was used for the SRT and 14.8 °C for the temperature, as measured in the Breda WWTP (October 2013 - July 2014). To correct for the difference between catabolic and total substrate removal (i.e., removal by catabolism and incorporation to sludge biomass), the yield factors were expressed as g $COD_{biomass}$ g^{-1} [COD or $N]_{catabolized}$ by calculating $Y \times (1 - Y)^{-1}$ in the case of COD and $Y \times (1 - 0.0875 \times Y)^{-1}$ in the case of N. Sludge biomass was assumed to have the empirical formula of $C_5H_7O_2N$ (Hoover & Porges, 1952), with a COD/VSS ratio of 1.42 and a N/COD ratio of 0.0875. The VSS/TSS ratio of the sludge was assumed to be 0.77 (Breda WWTP, October 2013 - July 2014).

2.3.4 Side stream treatment

Based on measurements in the Breda WWTP (October 2013 - July 2014), it was assumed that settled sludge from the primary and secondary stages had a constant biomass concentration of 9 g TSS L^{-1}, from which the flow rates in the side stream could be calculated. The anaerobic digestibility of the sludge from the primary settling, HiCAS and HiCS treatments was 0.59 g $COD_{removed}$ g^{-1} COD_{fed}, and was calculated as (0.67×T + 36)/100 (Van Haandel & van der Lubbe, 2007), where the digestion temperature was 35°C. In the scenarios where $FeCl_3$ was added as a coagulant, the digestibility of the primary sludge was decreased by 10% (Gossett et al., 1978). The digestibility of secondary sludge was 0.39 g $COD_{removed}$ g^{-1} COD_{fed}, and was calculated as $(a \times (0.67 \times T + 36) + (1-a) \times (0.19 \times T + 10))/100$, where the active fraction a was assumed to be 0.3 for a secondary sludge with SRT above 30 d (Van Haandel & van der Lubbe, 2007). The anaerobic sludge yield was assumed to be 0.05 g COD_{sludge} g^{-1} $COD_{removed}$, resulting in a methane yield of 0.95 g $COD_{methane}$ g^{-1} $COD_{removed}$. Methane has an energy content of 3.86×10^{-3} kWh g^{-1} COD, and the electrical efficiency during combustion of the biogas in a combined heat and power (CHP) installation was assumed to be 0.38 kWh_{el} $kWh_{methane}^{-1}$.

Digestion was assumed to be complete; i.e., the digestate contained only the non-digestible fraction of the feed sludge (consisting of COD_{part} and N_{part}), anaerobic biomass produced, and N_{diss} released by the digestible fraction of the feed sludge minus N_{diss} uptake due to anaerobic biomass growth. The VSS and TSS of the digestate were calculated by assuming that the COD/VSS ratio of the sludge streams was homogenous (i.e., the VSS digestibility in units of g $VSS_{removed}$ g^{-1} VSS_{fed} equaled the COD digestibility), and that the inorganic TSS was unaffected during digestion. The digestate was dewatered in a centrifuge with a capture of 80% of COD_{part} and N_{part} (Metcalf & Eddy, 2003) and dewatered sludge concentration of 20% DW prior to disposal. In the scenarios without side stream N removal, the reject water of the centrifuge was returned to the inlet of the main stream treatment. In the scenarios with side stream N removal, the reject water was subjected to PN/A treatment, assuming that incoming dissolved reduced nitrogen was removed for 85% and oxidized to nitrate for 7% (Lackner et al., 2014), resulting in 8% residual ammonia after treatment. The observed yield of the PN/A sludge was calculated as described in Section 2.3.3, for a reactor temperature of 30 °C. It was assumed that no COD or N_{part} was removed during side stream PN/A treatment. The produced PN/A sludge was dewatered to 20% DW for disposal. Upon recycling of the treated side stream water to the plant's main stream, the nitrate was denitrified in the primary stage (HiCAS and HiCS scenarios) or the secondary stage (other scenarios), thereby offsetting part of the oxygen demand. The residual ammonia and N_{part} of the side stream water was removed alongside the plant's influent nitrogen, as described in Sections 2.3.2 and 2.3.3.

2.3.5 Cost analysis

2.3.5.1 OPEX

For OPEX calculations, energy costs for aeration and non-aeration (pumping, settling and sludge dewatering) were considered, as well as the cost of coagulant and methanol dosage and sludge disposal. Energy saved from the combustion of biogas was subtracted from the gross energy costs. The industrial electricity price was assumed to be € 0.12 kWh^{-1}, which was the average industrial electricity price of the 28 EU countries in 2014 (Eurostat, 2015). Aeration requirements were calculated from the stoichiometric oxygen demand of 1, 4.57 and 3.43 g O_2 per gram of catabolic oxidation of COD, NH_4^+-N to nitrate and NO_2^--N to nitrate, respectively, and the stoichiometric saving of 2.86 and 1.71 g O_2 per gram of NO_3^--N and NO_2^--N reduced to N_2, respectively. The aeration requirement for PN/A was 1.81 g O_2 g^{-1} NH_4^+-N, as determined for the overall PN/A reaction (Barnes & Bliss, 1983; Strous et al., 1998). The energy demand for aeration was calculated from the oxygen transfer efficiency of 3.05 kg O_2 kWh^{-1} for both the primary and secondary stage, which was a result of the aeration conditions and the installed aeration system (see Section 2.3.5.2). Pumping energy was 0.02 kWh PE^{-1} d^{-1} (Siegrist et al., 2008). The energy demand for the combined primary and secondary settlers was estimated as 5.31 $\times 10^{-3}$ kWh $m^{-3}_{influent}$. The energy demand for dewatering was assumed to be 0.15 kWh kg^{-1} COD. Sludge disposal costs were € 320 ton^{-1} DW (Paul et al., 2006). The

coagulant price was € 427 ton^{-1} $FeCl_3$, including taxes and shipping (Breda WWTP, personal communication). The methanol price was € 150 ton^{-1} COD (Methanex, 2016).

2.3.5.2 CAPEX

For CAPEX calculations, only those costs were considered to upgrade an existing WWTP with secondary treatment to a two-stage system with primary and secondary treatment; i.e., only CAPEX costs for the construction of primary treatment tanks and primary settlers were considered. Construction costs were calculated at a detailed level, including costs for concrete basins, foundation, aeration infrastructure, sludge waste and sludge return pumps, and internal piping (i.e., piping up to 1 m outside the tank). Costs for structural calculations were included, as well as engineering (10 to 15% for a single reactor or settler unit) and profit/risk (15% of total CAPEX). Calculations were based on current industrial prices. Reactor basins for the HiCAS and HiCS systems were dimensioned on the combined peak influent flow rates and recycle flow rate, which was assumed to be constant at 50% of the average influent flow rate. Reactors were round, with a wall thickness of 30 cm, a bottom thickness of 25 cm and a 6 m water depth. The volume was calculated to result in a contact time (HRT_{act}) during peak flow of 46 min in the HiCAS system and a contact time of 15 min and stabilization time of 40 min in the HiCS system, as was the case in the laboratory experiments. Since the peak flow rate differed from the average flow rate by a factor 6, the reactors are over-dimensioned during average conditions.

Foundation piles (7 m, 25×25 cm) were placed every 4 m^2 of bottom surface, and foundation costs included costs for placement and finishing. The fine bubble disc aeration systems were designed for a 650 mbar pressure difference at 6 m water height, a sludge concentration of 3 g MLVSS L^{-1}, a DO set point of 2 g L^{-1} and a water temperature of 20°C. This resulted in an alpha factor of 0.7, beta factor of 0.78, and a specific oxygen transfer capacity of 19 g O_2 m^{-3} air m^{-1} water depth, leading to a final ratio of oxygen supplied/transferred of 4.82. Under these conditions, a 110 kW blower was assumed to operate at 80% capacity when transferring 12.9 ton O_2 d^{-1}, which corresponds to an electrical oxygen transfer efficiency of 3.05 kg O_2 kWh^{-1}. This efficiency is in the lower range of typical efficiencies for fine bubble disc aeration systems (Xylem, 2012), and was used as a constant for OPEX calculations (see Section 2.3.5.1). The number of aeration discs and the required blower capacity were dimensioned based on the oxygen demand in the HiCAS and HiCS scenarios without chemical coagulation and with nitrogen removal by nit/denit. In the HiCS contactor, where no aeration system was present, a submersible mixer was included, with slow rotator and tripod support. Primary settlers were dimensioned for an overflow velocity of 24 m d^{-1} during average flow conditions and had a height of 3 m with a water height of 2.5 m. The number of settlers was chosen so that the diameter per settler did not exceed 50 m. Sludge return pumps were installed as parallel screw pumps with a capacity of 32000 m³/d each, a pumping height of 6 m and an angle of 30°. Sludge waste pumps were installed as centrifugal pumps with a capacity of 7200 m³/d, and a pumping height of 5 m. The CAPEX costs were amortized at an interest rate of 5.6% over a period of 20 years.

2.4 Sensitivity analysis

A local sensitivity analysis was performed on the HiCAS scenario with addition of chemical coagulant, nitrogen removal by nit/denit, and side stream PN/A treatment. This scenario was selected because it required addition of methanol for denitritation in the secondary stage, and this allowed evaluating the effect of methanol costs on the overall OPEX. In the scenario for sensitivity analysis, 7 mg L^{-1} of NO_3^--N was artificially added to the final effluent, so that the effluent N concentration was slightly above the maximum value and additional denitrification was needed. This allowed evaluating the effect of the required effluent quality on plant OPEX. A total of 33 parameters and variables, as used in the scenario assumptions, were evaluated through local sensitivity analysis. To keep the COD balance in the primary stage always at 100%, the scenario was built in such a way that any change in the percentage of COD redirected to waste would by result in different percentages of COD in the effluent but not affect the percentage of COD oxidized, and vice versa. The sensitivity of the scenario to a given parameter was evaluated as the absolute value of the increase or decrease in OPEX by varying the given parameter between -3.3% and +3.3% of their original value, divided by the OPEX under unchanged conditions.

3 Results

3.1 Reactor and batch experiments

Reactor experiments were performed to evaluate difference in reactor performance, and assess the repeatability / reversibility of switching between the two systems. Operational details for the reactor experiments are presented in **Table 5.1**. During HiCAS operation, the reactor removed 68±16 % of COD_{tot}. When the reactor was switched to HiCS operation, the COD_{tot} removal efficiency decreased to 47±14 %, and was restored again to 72±7 % when the reactor was switched back to HiCAS operation. However, the fraction of incoming COD recovered as waste sludge remained comparable at percentages of 39±7 %, 43±17 % and 47±8 % for the HiCAS, HiCS and reverted HiCAS configuration, respectively. The main difference between HiCAS and HiCS operation was the lower oxidation percentage in the HiCS reactor, which caused more COD to leave the system through the effluent. The extent of oxidation decreased from 33±24 % in HiCAS operation to 7±16 % in HiCS operation, and increased again to 27±23 % in the reverted HiCAS operation. In the batch tests for coagulant dosage, the optimal dosage of $FeCl_3$ was determined as 0.28, 0.041 and 0.065 g $FeCl_3$ g^{-1} $COD_{part+coll}$ of the influent, the HiCAS and the HiCS sludge, respectively. The COD balances for all primary treatment technologies are shown in **Figure 5.1**.

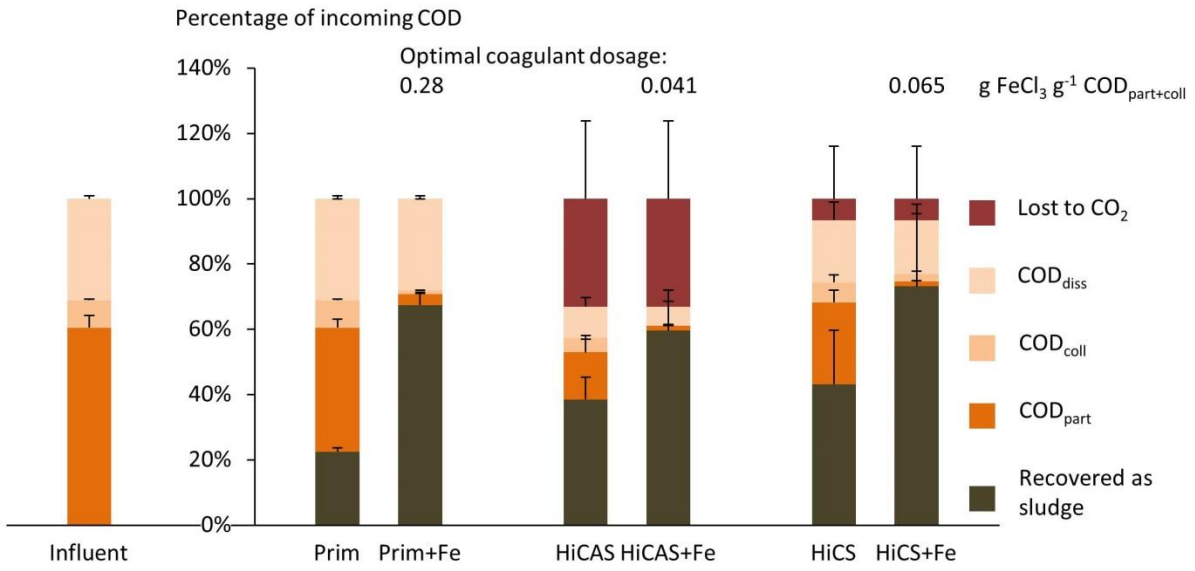


Figure 5.1: COD balances for the primary treatment technologies, with indication of the optimal coagulant dosage. The COD balance for primary settling ('Prim') was calculated from Siegrist et al. (2008). The COD balances for the HiCAS and HiCS systems were determined from the reactor experiments (n = 5 to 6). The shifts in COD balances due to the addition of coagulant were calculated from the coagulant batch tests.

Removal of nitrogen occurred primarily through N_{part} removal, which was 56% in the HiCAS and 33% in HiCS operation. Removal of N_{diss} was negligible in both reactor configurations (**Table 5.1**).

3.2 Scenario analysis

3.2.1 Carbon and nitrogen removal

Figure 5.2 A shows the outgoing COD balance for all biological scenarios through the different pathways of oxidation in the primary treatment, aerobic and anoxic oxidation in the secondary treatment, removal via disposed sludge, conversion to methane, and washout in the effluent. In the scenarios with HiCAS as primary treatment and N/DN as secondary treatment, the outgoing COD load was higher than the incoming load, because they required addition of methanol for nitrogen removal.

Figure 5.2 B shows the N balance, divided along the different removal pathways of N/DN, nit/denit, or PN/A, sludge disposal, and washout via the effluent. In all scenarios, the major fraction of N was catabolically converted to N_2 via one of the three removal technologies. The scenarios with PN/A in the secondary treatment had a higher effluent N concentration, because of the production of nitrate as an end product of the anammox reaction.

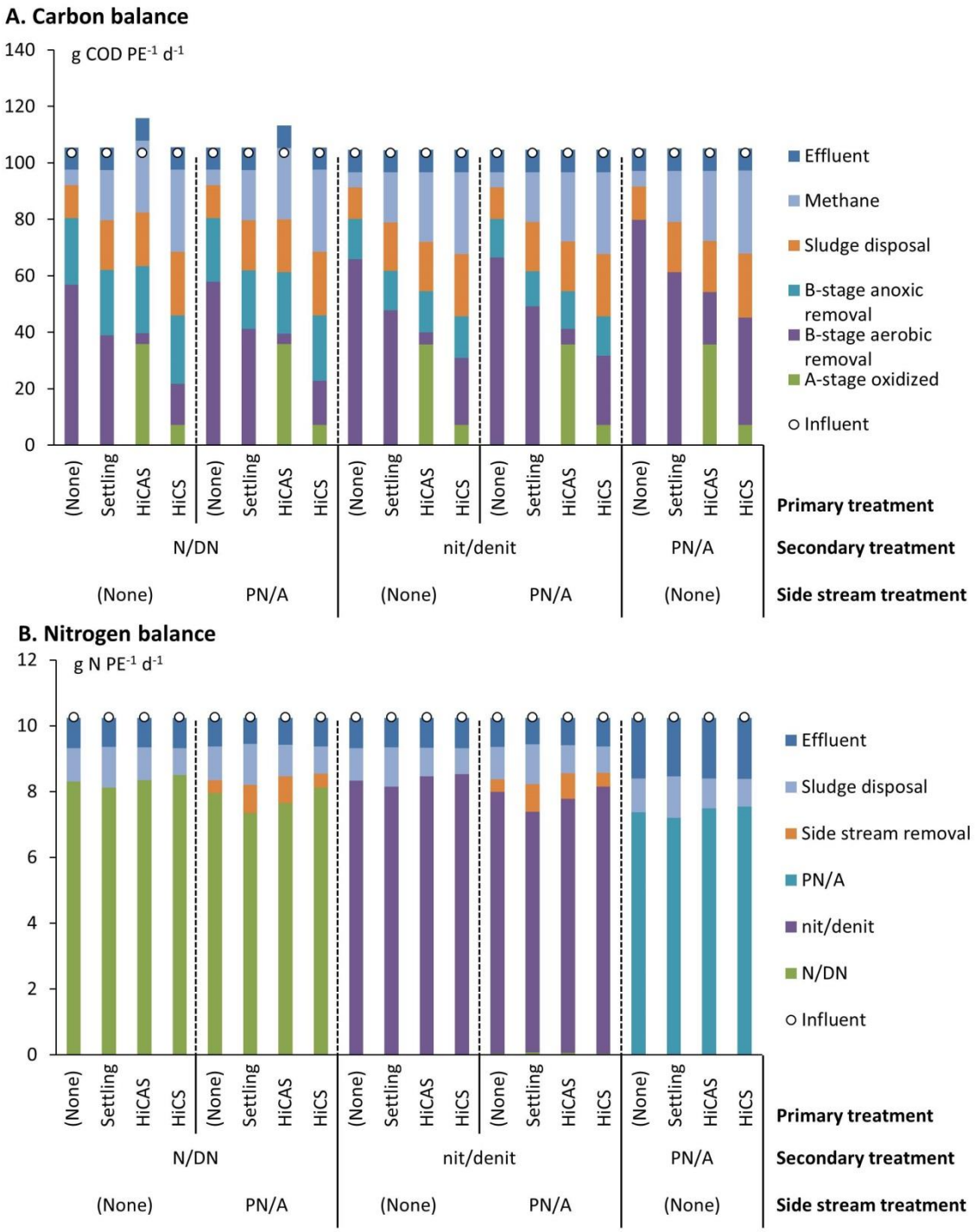


Figure 5.2: (A) COD and (B) nitrogen balance over all biological scenarios. White dots show the level of influent COD and nitrogen, respectively. Scenarios are sorted alongside their primary treatment technology (none, primary settling, HiCAS or HiCS), technology for secondary N removal (N/DN, nit/denit, or PN/A), and technology for side stream N removal (none or PN/A). PE: population equivalents.

The effluent nitrogen concentration for the biological scenarios is given in **Table 5.3**. Depending on the scenario, effluent N concentrations varied between 3.7 and 4.3 mg N L^{-1} for the scenarios with N/DN and nit/denit, and up to 8.7 mg N L^{-1} for the scenarios with PN/A. In none of the scenarios, the effluent nitrogen concentration exceeded the predefined maximum of 10 mg L^{-1}. In case of more stringent discharge limits, some scenarios might require extra carbon addition for denitrification prior to discharge.

Table 5.3: Effluent N concentrations for the different scenarios, where all N_{eff} was assumed to be nitrate. N.a: not applicable.

	Secondary treatment:				
	N/DN		nit/denit		PN/A
	Side stream treatment:				
Primary treatment:	(none)	PN/A	(none)	PN/A	(none)
(none)	4.33	4.12	4.34	4.14	8.63
Primary	4.17	3.74	4.19	3.76	8.38
HiCAS	4.24	3.85	4.30	3.92	8.66
HiCS	4.31	4.10	4.33	4.12	8.71

3.2.2 Energy consumption and OPEX

The energy balance of the biological scenarios is given in **Figure 5.3 A**. Expressed as annual cost per population equivalent (PE), the scenarios without primary treatment had the highest net energy consumption (> 17 kWh PE^{-1} y^{-1}), while the lowest net energy consumption was achieved in the HiCS scenarios (from 0.98 kWh PE^{-1} y^{-1} in the scenario with PN/A as secondary treatment to 1.1 kWh PE^{-1} y^{-1} in the HiCS scenarios with nit/denit). **Figure 5.3 B** shows the OPEX balance of all scenarios. The HiCS scenarios had the lowest OPEX, with only slight differences between the secondary nitrogen removal technologies (from € 2.91 PE^{-1} y^{-1} in the scenario with nit/denit to € 2.97 PE^{-1} y^{-1} in the scenario with secondary PN/A), followed by the HiCAS scenarios with secondary treatment by nit/denit and PN/A (€ 2.98 to 3.04 PE^{-1} y^{-1}). The two most expensive scenarios were the ones with HiCAS + N/DN and HiCAS + N/DN + side stream PN/A (€ 3.81 and 3.61 PE^{-1} y^{-1}, respectively).

In general, energy consumption and OPEX decreased as the primary treatment progressed from no treatment, over primary settling and HiCAS, to HiCS. The choice for primary treatment technology had a stronger impact on the plant-wide energy and cost balances than secondary and side stream treatment technologies. The secondary treatment options only had a considerable impact on OPEX in case HiCAS was used as primary treatment, because HiCAS removed so much COD that addition of an external carbon source was required in case of nitrogen removal by N/DN. The disadvantageous effect of combining HiCAS with secondary N/DN was slightly alleviated when side stream PN/A was

used, because this allowed a larger fraction of the nitrogen to be removed without methanol addition.

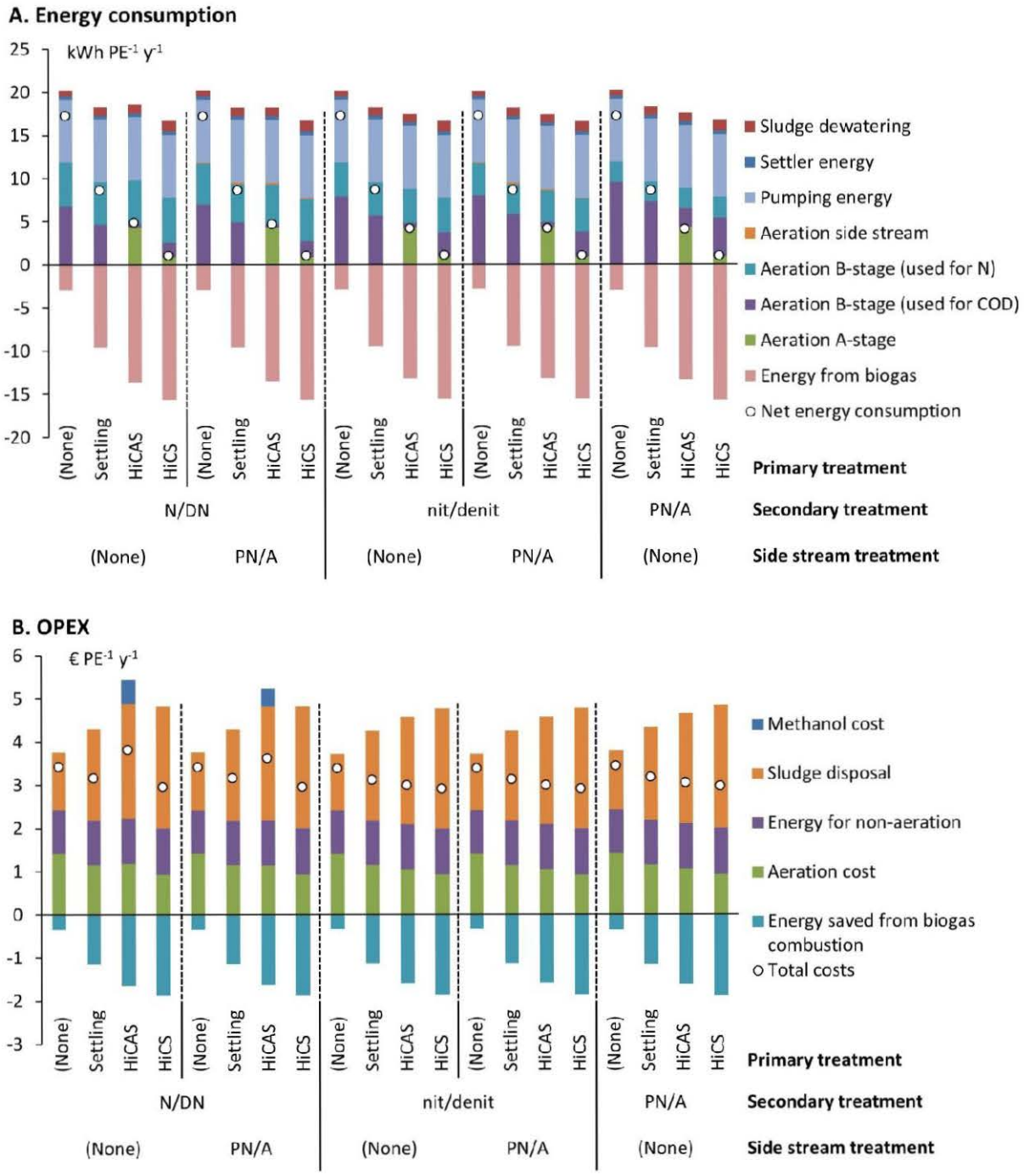


Figure 5.3: (A) energy consumption and (B) OPEX balance over all biological scenarios. Black dots show the net energy consumption and net OPEX, respectively. Scenarios are sorted alongside their primary treatment technology (none, primary settling, HiCAS or HiCS), technology for secondary N removal (N/DN, nit/denit, or PN/A), and technology for side stream N removal (none or PN/A). PE: population equivalents.

3.2.3 Chemical coagulation

For the scenarios with primary settling, HiCAS and HiCS as primary treatment, additional scenarios were calculated where $FeCl_3$ was added as coagulant to improve COD recovery. **Figure 5.4** shows the outgoing COD balance and N balance of these scenarios. Coagulant addition substantially improved the overall COD balance of the plant. When $FeCl_3$ was added to the primary settling, HiCAS or HiCS stage, methane production accounted for 32% to 42% of incoming COD. In comparison, without $FeCl_3$ addition (compare to **Figure 5.2**), this was only 17% to 28% of incoming COD, not considering the scenarios where no primary treatment was present. However, when HiCAS and HiCS were combined with addition of $FeCl_3$ and secondary N/DN or nit/denit, considerable amounts of external COD needed to be added (4% to 23% of influent COD), because the improved COD recovery did not leave sufficient COD in the primary effluent to allow secondary nitrogen removal. External COD addition was not required in the scenarios where $FeCl_3$ was combined with primary settling, or in the scenarios with secondary PN/A.

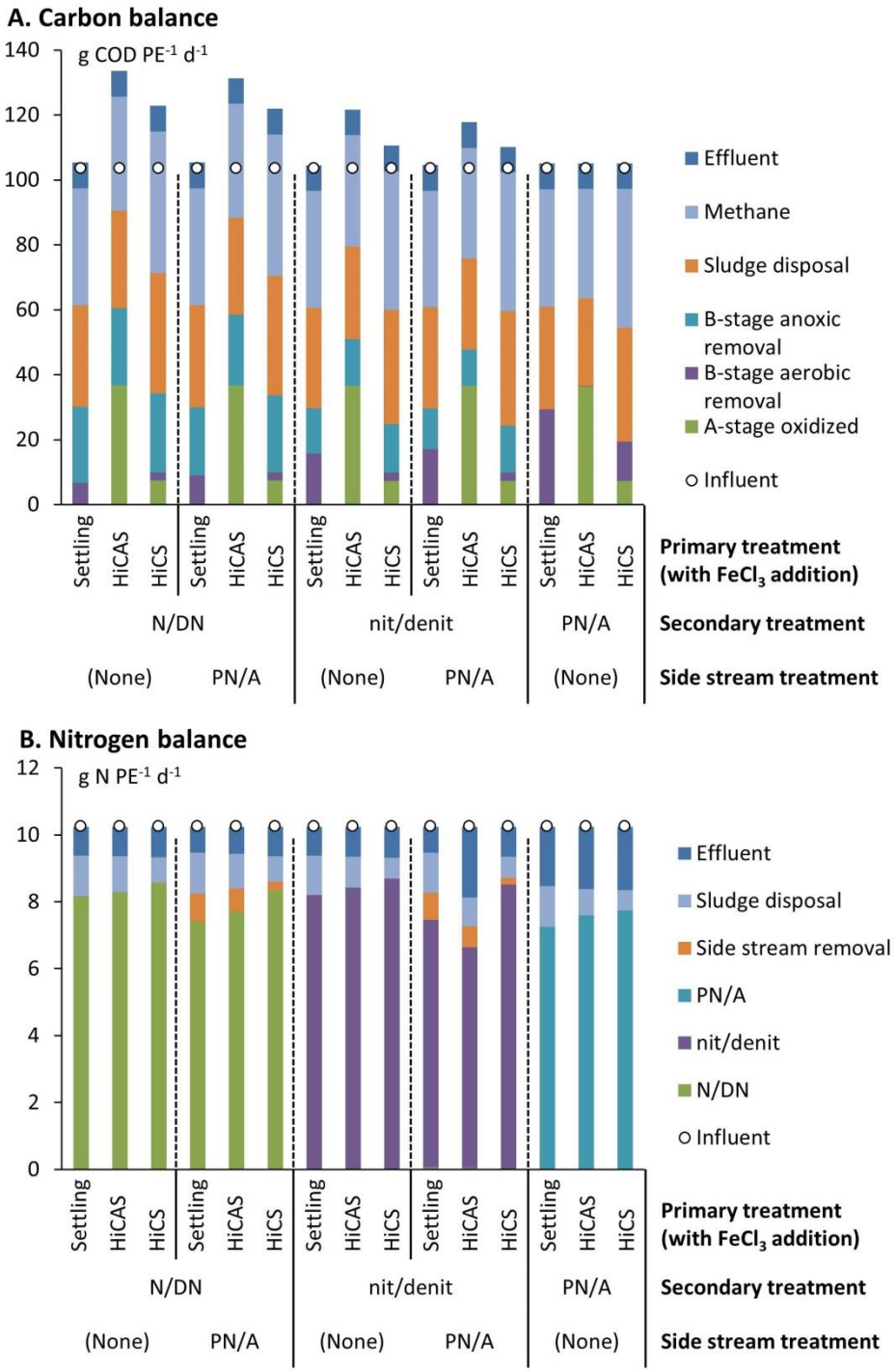


Figure 5.4: (A) COD and (B) nitrogen balance over all scenarios with chemical coagulation. White dots show the level of influent COD and nitrogen, respectively. Scenarios are sorted alongside their primary treatment technology (none, primary settling, HiCAS or HiCS), technology for secondary N removal (N/DN, nit/denit, or PN/A), and technology for side stream N removal (none or PN/A). PE: population equivalents.

The energy and OPEX balances for the scenarios with chemical coagulation are shown in **Figure 5.5**. All scenarios were energy positive, with the energy produced from biogas combustion covering between 101% and 159% of gross energy consumption. The HiCS scenarios were most energy positive, with an energy recovery of 145% to 159% of gross consumption, depending on the secondary and side stream technology. The HiCAS scenarios were least energy positive, with recovery percentages between 101% and 113%, because of the relatively high fraction of COD oxidation in the HiCAS process and the associated decrease in biogas recovery. The OPEX calculations show that scenarios with chemical coagulation are between 38% and 155% more expensive than their corresponding scenarios without chemical coagulation (compare to **Figure 5.3**), primarily due to increased costs for sludge disposal, and the costs for coagulant and methanol addition. The scenarios with HiCS as primary treatment technology were generally less expensive than those with primary settling or HiCAS, except when PN/A was used during secondary treatment. In the latter case, HiCAS was the least expensive technology because it required a slightly lower dosage of coagulant. The importance of coagulant dosage on the overall OPEX, and thus of further optimization of the coagulation process, was most clearly demonstrated by the high OPEX in the scenarios with primary settling, which were 153% to 155% more expensive with chemical coagulation compared to primary settling scenarios without chemical coagulation.

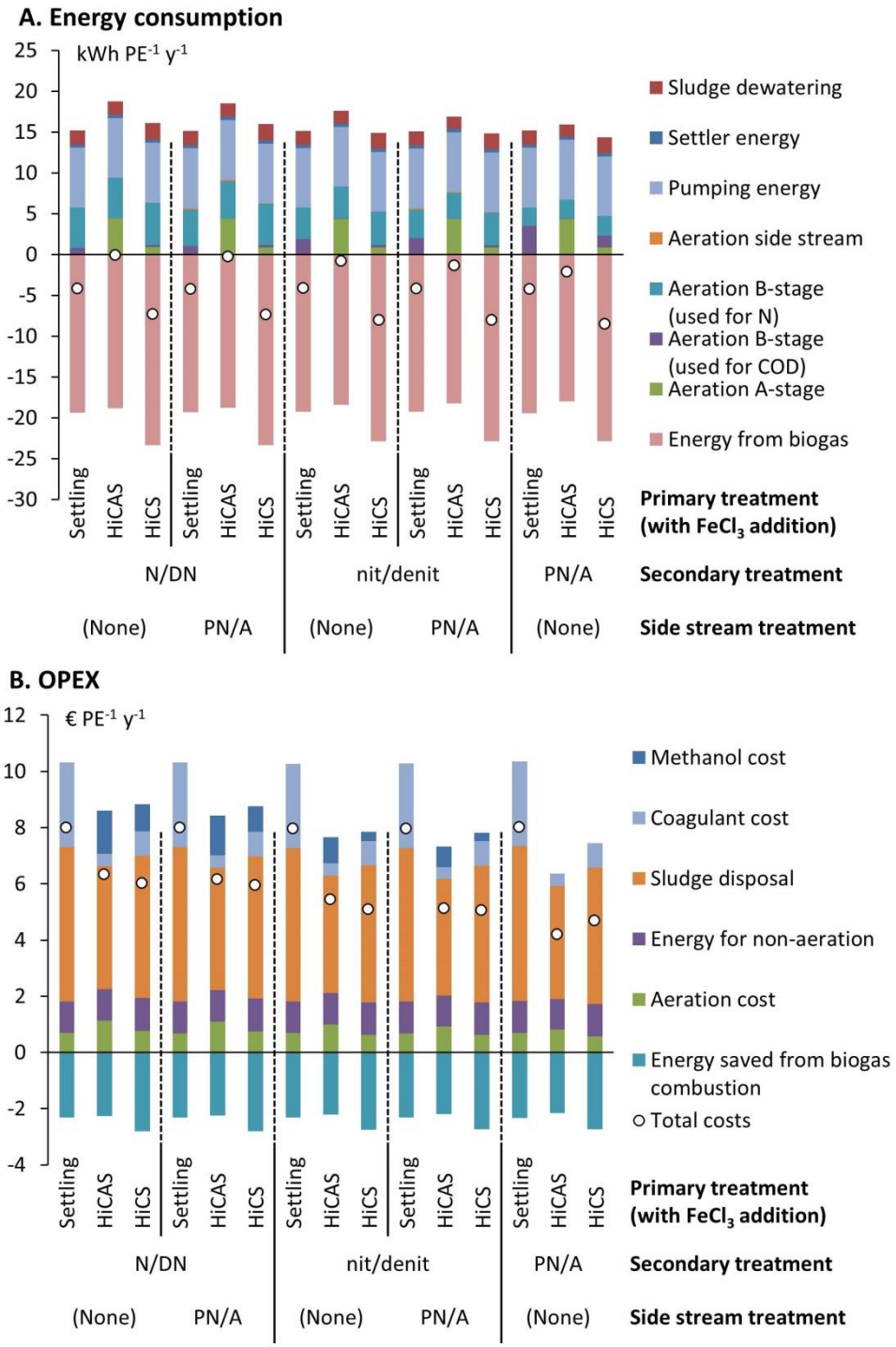


Figure 5.5: (A) energy consumption and (B) OPEX balance over all scenarios with chemical coagulation. Black dots show the net energy consumption and net OPEX, respectively. Scenarios are sorted alongside their primary treatment technology (none, primary settling, HiCAS or HiCS), technology for secondary N removal (N/DN, nit/denit, or PN/A), and technology for side stream N removal (none or PN/A). PE: population equivalents.

3.3 Sensitivity of the model

The results of the local sensitivity analysis are shown in **Figure 5.6**.

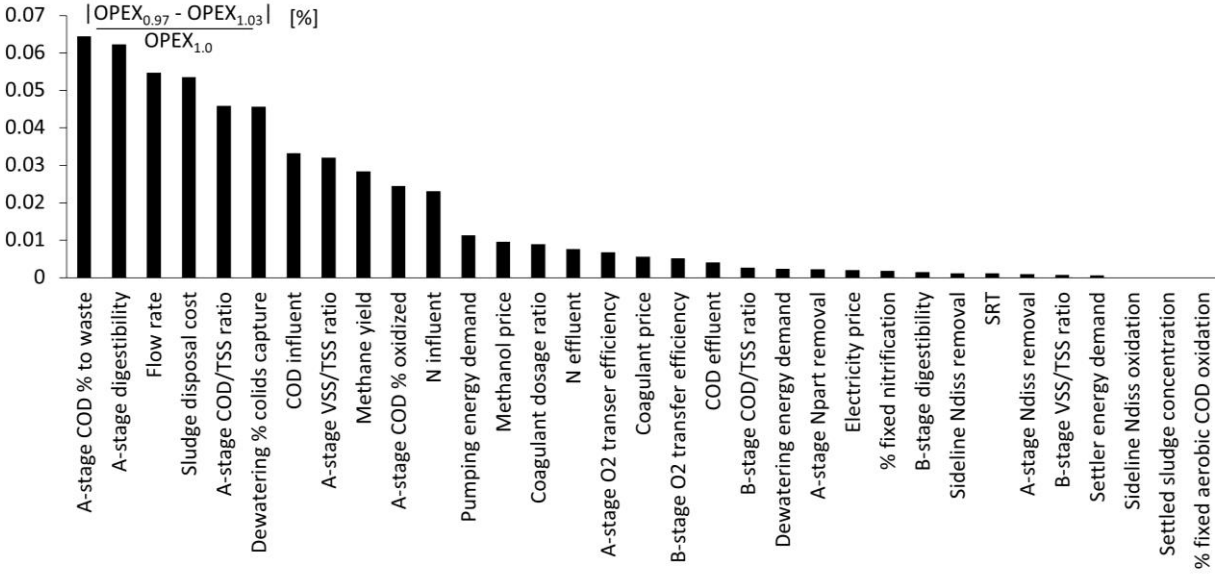


Figure 5.6: Local sensitivity analysis for parameters and variables of the scenario analysis. The effect on total OPEX was evaluated as the absolute value of the increase or decrease in OPEX by varying each parameter between -3.3% and +3.3% of their original value, divided by the OPEX under unchanged conditions (%).

The parameters with the strongest influence on plant operational costs, not counting the influent characteristics, were (1) the percentage of COD redirected to waste in the primary stage, (2) the anaerobic digestibility of the primary sludge, (3) the costs for sludge disposal, (4) the COD/TSS ratio of the primary sludge, (5) the percentage of solids capture during sludge dewatering, (6) the VSS/TSS ratio of the primary sludge, (7) the COD-to-methane conversion yield, and (8) the percentage of COD oxidized in the primary stage.

3.4 CAPEX for retrofitting a two-stage installation

An estimation of investment costs was made for expanding an existing stand-alone CAS installation to a two-stage plant with a primary treatment stage consisting of a primary settling, HiCAS or HiCS system. In each of these three cases, two primary settlers were factored in. To obtain the required HRT, the total volume requirement of the HiCS reactors was 61% lower than that of the HiCAS reactor. Detailed CAPEX calculations are summarized in **Table 5.4**.

Table 5.4: Investment costs for construction of a primary settling, HiCAS or HiCS system for primary treatment. Costs are expressed as total CAPEX, CAPEX per thousand population equivalents (kPE) and amortized costs per kPE, with an interest rate of 5.6% over a period of 15 years.

CAPEX elements									€	€ kPE^{-1}	€ kPE^{-1} y^{-1}
Contactor tank (HiCAS)											
Reactor basin and internal piping	16804	m³	x	31	€/m³	x	1	=	€ 520 936	€ 1 388	€ 117
Foundation piles	701	piece	x	290	€/piece	x	1	=	€ 203 290	€ 542	€ 46
Structural calculations									€ 154 132	€ 411	€ 35
Screw pump sludge return	2	piece	x	44333	€/pump	x	1	=	€ 88 667	€ 236	€ 20
Aeration blower 110 kW				37000	€/piece	x	2	=	€ 74 000	€ 197	€ 17
Aeration pipes to reactor (DN 300)	9	m	x	1167	€/m	x	2	=	€ 21 000	€ 56	€ 4.7
Aeration discs	1794	piece	x	40	€/disc	x	2	=	€ 143 520	€ 382	€ 32
Engineering for basin				15%					€ 180 832	€ 482	€ 41
Contactor tank (HiCS)											
Reactor basin and internal piping	5480	m³	x	39	€/m³	x	1	=	€ 213 708	€ 569	€ 48
Foundation piles	229	piece	x	290	€/piece	x	1	=	€ 66 410	€ 177	€ 15
Structural calculations									€ 21 146	€ 56	€ 4.8
Engineering for basin				15%					€ 45 190	€ 120	€ 10
Mixer, submersible, slow rotator	1	piece	x	17800	€/piece	x	1	=	€ 17 800	€ 47	€ 4.0
Engineering for mixer				10%					€ 1 780	€ 4.7	€ 0.4
Stabilizer tank (HiCS)											
Reactor basin and internal piping	1113	m³	x	54	€/m³	x	1	=	€ 60 075	€ 160	€ 14
Foundation piles	47	piece	x	290	€/piece	x	1	=	€ 13 630	€ 36	€ 3.1
Structural calculations									€ 4 126	€ 11	€ 0.9
Screw pump sludge return	2	piece	x	44333	€	x	1	=	€ 88 667	€ 236	€ 20
Aeration blower 55 kW				22000	€/piece	x	1	=	€ 22 000	€ 59	€ 4.9
Aeration pipes to reactor (DN 200)	9	m	x	939	€/m	x	1	=	€ 8 450	€ 23	€ 1.9
Aeration discs	361	piece	x	40	€/disc	x	1	=	€ 14 440	€ 38	€ 3.2
Engineering for basin				15%					€ 31 708	€ 84	€ 7.1
Settler (Primary settling, HiCAS, HiCS)											
Settler basin and internal piping	5006	m³	x	40	€/m³	x	2	=	€ 400 500	€ 1 067	€ 90
Foundation piles	418	piece	x	290	€/piece	x	2	=	€ 242 440	€ 646	€ 55
Structural calculations									€ 7 530	€ 20	€ 1.7
Engineering for settler basin				15%	for 1 unit				€ 49 350	€ 131	€ 11
Radial scraper system	23	m	x	2600	€/m	x	2	=	€ 119 846	€ 319	€ 27
Trough	145	m	x	380	€/m	x	2	=	€ 110 056	€ 293	€ 25
Engineering for scrapers				10%	for 1 unit				€ 11 495	€ 31	€ 2.6
Centrifugal waste pump (HiCAS, HiCS)	1	piece	x	9830	€/piece	x	2	=	€ 19 660	€ 52	€ 4
Engineering waste pump				15%	for 1 unit				€ 2 949	€ 8	€ 1
Overall CAPEX											
Subtotal primary settling									€ 941 218	€ 2 508	€ 212
Profit / risk				15%					€ 141 183	€ 376	€ 32
Total primary settling									**€ 1 082 401**	**€ 2 884**	**€ 243**
Subtotal HiCAS									€ 2 350 203	€ 6 262	€ 528
Profit / risk				15%					€ 352 530	€ 939	€ 79
Total HiCAS									**€ 2 702 733**	**€ 7 202**	**€ 608**
Subtotal HiCS									€ 1 572 956	€ 4 191	€ 354
Profit / risk				15%					€ 235 943	€ 629	€ 53
Total HiCS									**€ 1 808 899**	**€ 4 820**	**€ 407**

The investment costs were lowest for the primary settling system, with a CAPEX of € 243 per thousand population equivalents (kPE) per year. Costs for a HiCS + settler system were intermediate (€ 407 kPE^{-1} y^{-1}), and costs for a HiCAS + settler system were the highest (€ 608 kPE^{-1} y^{-1}).

4 Discussion

4.1 Switching configurations between HiCAS and HiCS

The reactor experiments demonstrated that an operational switch from a conventional A-stage (HiCAS configuration) to a HiCS configuration resulted in a notable decrease in the amount of COD oxidized, and higher COD concentrations in the effluent instead. These changes were completely reverted when reactor operation was switched to HiCAS configuration again. This indicates that the reactor configuration was likely the sole cause of the observed changes in COD balance, and that a configuration switch from HiCAS to HiCS will result in a predictable and repeatable change in performance – a prerequisite for full-scale implementation of the HiCS process.

The synthetic wastewater used in these reactor experiments had a strength of 460 mg COD L^{-1}. This corresponds to a medium-strength domestic wastewater (Metcalf & Eddy, 2003), and is about half the strength of the influent that was used in previous HiCS experiments (Chapters 3 and 4). While the HiCS reactor in this study achieved a COD recovery of 43±17 % and a 50±12 % COD washout through the effluent, a HiCS reactor under nearly identical conditions but an influent strength of 800 mg COD L^{-1} was able to achieve a higher COD recovery (55±10 %) and lower COD washout (31±10 %) (Chapter 4). These differences may be due to the relatively large error on the reported values, but could also indicate an influence of influent strength and/or composition on the performance of the HiCS system. It is recommended that the HiCS process be optimized towards the specific influent characteristics of a given facility before full-scale implementation (see Chapter 6, Section 3.1).

4.2 Methodology evaluation

The scope of this work was to evaluate the impact of different operational strategies for COD and N removal on the OPEX costs of wastewater treatment, and provide an estimate of the investment costs necessary to expand an existing stand-alone CAS installation to a two-stage system. This study did not incorporate all factors of CAPEX and OPEX costs. For a full estimation of wastewater treatment costs, additional factors should be included, such as maintenance and salaries, investment costs for control systems and small electronics, chemical dosage systems, ground works, buildings, piping, cabling, etc.

Phosphorus removal was not addressed in this study. The reported OPEX only represents costs for COD and N removal. WWTPs may experience additional operational and investment costs for P removal, depending on the technology applied (e.g., chemical precipitation, biological accumulation). Pumping costs were assumed the same for all scenarios. The addition of a primary treatment stage to a CAS installation may create extra pumping costs, because of additional recirculation streams. On the other hand, it can be expected that internal MLSS recirculation can be lowered when optimized nitrogen removal technologies are applied (Kartal et al., 2010), resulting in lower pumping costs. It was outside the scope of this work to provide an accurate estimation of the changes in overall plant pumping costs. The CAPEX for the primary settlers was the same in all scenarios. Settlers all had an

equal size, because they were designed for the same overflow velocity of 24 m d^{-1} in average flow rate conditions, which is in the lower range of the recommended 24 to 49 m d^{-1} for primary settlers (WEF, 2005). The amortization period of 20 years is a conservative period, given the fact that investment loans in the public water and wastewater sector are often amortized over a period of 25 - 30 years (CBO, 2002).

Many wastewater treatment plants around the world operate a primary settling stage and a secondary stage with N/DN, either with or without side stream PN/A. In this study, these scenarios had a net energy consumption of 8.65 and 9.61 kWh PE^{-1} y^{-1}, respectively, or about 0.11 kWh m^{-3} water treated. This is lower than the estimate of 16 kWh PE^{-1} y^{-1} by Siegrist et al. (2008), but similar to the estimate of 0.12 kWh m^{-3} wastewater by Scherson and Criddle (2014), for similar two-stage plant layouts. The OPEX costs for both these scenarios were estimated at € 3.15 PE^{-1} y^{-1}. Zessner et al. (2010) reported slightly higher costs for similar systems, with a total cost of € 18 PE^{-1} y^{-1} for WWTP plants with primary settling and N/DN (CAPEX + OPEX), of which 30-38% were operational costs. In the same study, costs for sludge treatment and disposal accounted for up to 40% of operational costs, which is in the same order as the 49% spent for sludge disposal in this study.

The costs for sludge disposal increased in those scenarios with a higher COD recovery (**Figure 5.3 B**). This may indicate that the digestibility of high-rate sludge was underestimated, since an increase in COD redirection to sludge should ideally lead to an increased methane production, not an increased amount of sludge to dispose. The energy demand for sludge dewatering and the solids content of dewatered sludge were assumed to be constant in all scenarios. However, the dewaterability of sludge may be affected by its type (primary or secondary) and by the addition of chemical coagulants. Given the strong influence of parameters associated with dewatering on the overall OPEX (**Figure 5.6**), it is recommended that more accurate information about sludge dewatering and disposal costs be collected during further studies.

In this study, chemical coagulation was responsible for a sharp increase in OPEX, because of the increased costs for chemicals and sludge disposal. The dosage of $FeCl_3$ in this study was based on preliminary batch experiments. Specialized studies on the optimization of factors such as pH, stirring time and stirring intensity during chemical coagulation in function of the desired COD redirection percentage, may show that chemical dosage can have a more favorable cost balance in modern AB-systems. Furthermore, by combining coagulants with polymeric flocculants, total dosage rates and costs may be significantly reduced (Bratby, 2006).

4.3 Selection of the optimal scenario

The highest operational costs were associated with scenarios where chemical coagulants were added to improve the solid/liquid separation in the primary stage (**Figure 5.5**). The increased overall costs were mainly associated with higher costs for sludge disposal, and to a lesser extent the cost of the $FeCl_3$ itself. Given the dosage rate assumed in this study, it is not economically attractive to perform chemical coagulation on a continuous basis. Systems without primary treatment (i.e., stand-alone

CAS systems) are in urgent need of optimization, as evidenced by the fact that these scenarios had the highest net energy consumption, as well as the highest overall OPEX of all scenarios without addition of coagulants or methanol. Therefore, a two-stage system is likely the most attractive approach to wastewater treatment.

The choice between N removal technologies in the secondary stage depends on the choice of the COD removal technology in the primary stage. As long as COD recovery in the primary stage is not fully optimized yet, the choice between nitrogen removal technologies will have little impact on plant operation. This can be seen in the scenarios with a primary settling or HiCS system. In these scenarios, the COD/N ratio of the primary effluent was 8.1 and 5.4 g g^{-1}, respectively, which is higher than the required 4.4 g g^{-1} for complete N removal via N/DN (including cell growth and 20% of aerobic COD oxidation) (Matějů et al., 1992). At such high COD/N ratios, energy consumption and OPEX were nearly identical for the different scenarios of secondary N removal, because COD removal simply shifted between anoxic and aerobic as the COD requirement for N removal changed. Of all scenarios without chemical coagulation, only the HiCAS scenarios were able to achieve a COD/N ratio in the primary effluent lower than the ratio required for N/DN, with values around 3.2 g g^{-1}. For HiCAS, as well as any other primary treatment technology, it becomes necessary to develop a reliable nit/denit or PN/A treatment as soon as the COD/N ratio of the primary effluent reaches a value below 4.4 g g^{-1}. In general, the presence or absence of side stream PN/A did not have a major impact on the overall energy and cost balance of the scenarios, since the fraction of nitrogen treated through side stream PN/A remained limited. Among the scenarios considered in this study, an optimal approach for wastewater treatment can be selected. Since nit/denit and mainstream PN/A are not fully developed yet, nitrogen removal needs to occur primarily through N/DN. Due to their high overall costs, the scenarios with chemical coagulation are not economically attractive. Since side stream nitrogen removal through PN/A did not have a major influence on the overall plant energy consumption and OPEX, the presence of a side stream PN/A system is not considered a priority when improving the energy and cost balance of a modern AB-system. Therefore, three scenarios can be proposed as potential alternatives to the stand-alone CAS scenario without chemical coagulation or side stream N removal, based on the primary treatment technology: primary settling, HiCAS (conventional A-stage), and HiCS. **Figure 5.7** compares the COD flow through the plant for each of these scenarios.

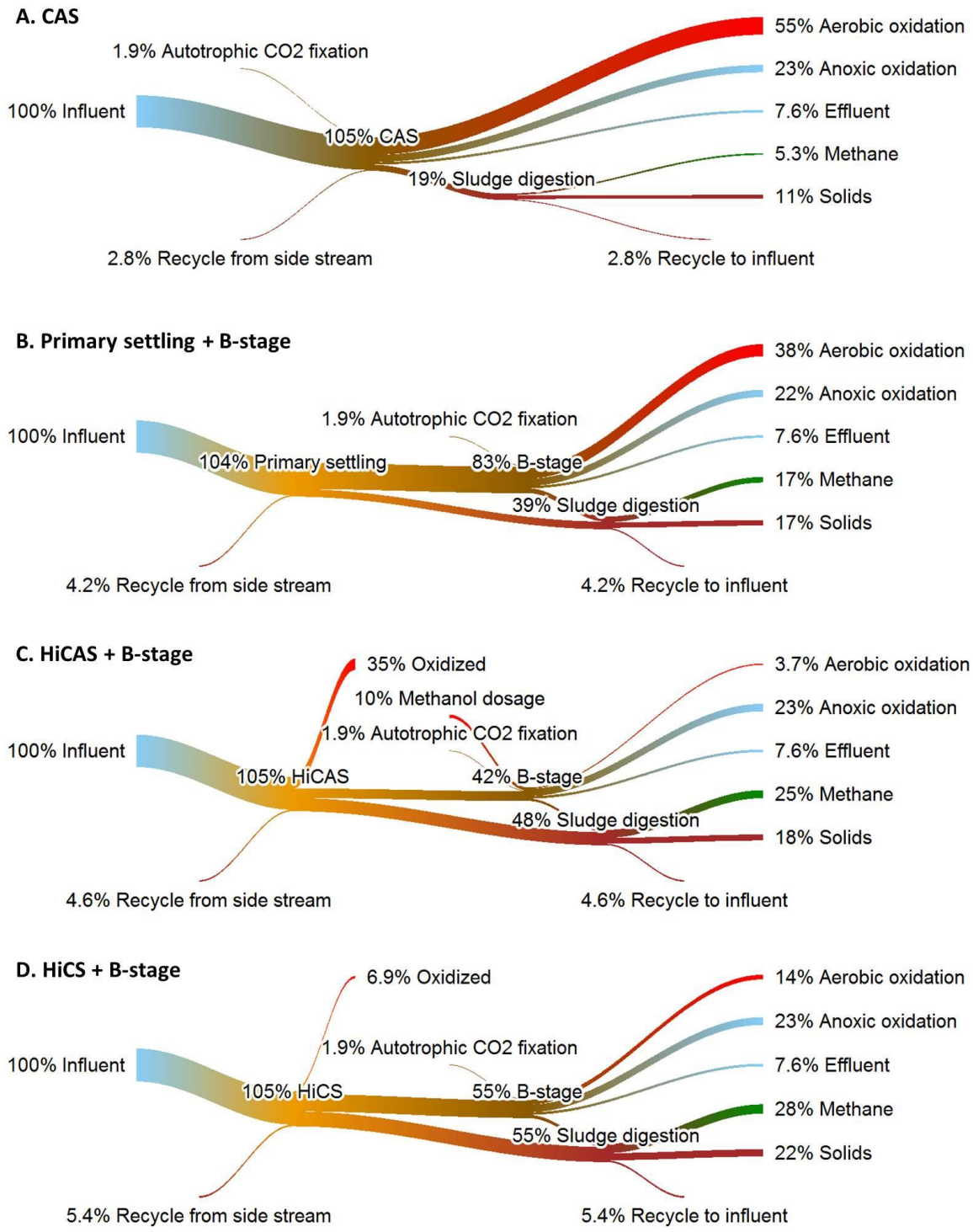


Figure 5.7: Sankey diagrams of the COD flow through (A) a stand-alone CAS scenario, and three two-stage scenarios with (B) primary settling, (C) HiCAS (conventional A-stage), and (D) HiCS as primary treatment stage. In all cases, nitrogen removal occurred by N/DN in the secondary treatment.

As can be seen in **Figure 5.7**, further optimization of the primary treatment stage is needed in each of the two-stage scenarios. The COD balances of the primary settling and HiCS systems need

optimization of the COD recovery percentage, given the large fraction of COD washing out to the effluent. Improving the COD redirection by means of chemical coagulation is likely not economically feasible at the dosage rates considered in this study, and optimization efforts should primarily focus on improving biological flocculation (see Chapter 6). In order to improve the solid / liquid separation performance in the HiCS system, further work should evaluate whether membrane filtration may be able to improve plant performance at reasonable costs. In contrast, optimization efforts for the HiCAS system need to focus on reducing the extent COD oxidation rather than improving biosorption, because the HiCAS effluent already contains COD concentrations too low to allow complete nitrogen removal by N/DN. In this case, further optimization of the COD balance is only relevant if technological advances allow reliable mainstream nit/denit or PN/A for nitrogen removal.

In **Table 5.5**, the net energy consumption and OPEX for these scenarios are presented, as well as the CAPEX needed to expand and existing stand-alone CAS installation with a primary treatment stage.

Table 5.5: Net energy consumption, CAPEX and OPEX for stand-alone CAS scenario and three two-stage scenarios with primary settling (Prim-B), HiCAS (conventional A-stage, HiCAS-B) and HiCS (HiCS-B) as primary treatment stage. In all cases, nitrogen removal occurred by N/DN in the secondary treatment, and no chemical coagulation or side stream PN/A was performed. PE: population equivalent.

	CAS N/DN	**Prim-B N/DN**	**HiCAS-B N/DN**	**HiCS-B N/DN**	
Net energy consumption	17.2	8.65	4.90	1.04	kWh PE^{-1} y^{-1}
OPEX	3.41	3.15	3.81	2.94	€ PE^{-1} y^{-1}
CAPEX (*)	0	0.24	0.61	0.41	€ PE^{-1} y^{-1}
CAPEX (*) + OPEX	3.41	3.40	4.42	3.35	€ PE^{-1} y^{-1}

*CAPEX is calculated as the investment costs needed to upgrade a stand-alone CAS installation to a two-stage system

In terms of the total CAPEX + OPEX costs, the HiCS-B system is the least expensive system, followed by the primary settling + B-stage system and the stand-alone CAS treatment. The conventional A-B system is most expensive. Considering these results, the economic gains might be worthwhile to convert existing stand-alone CAS or two-stage systems to HiCS-B systems. Since the HiCS-B system is the only of the scenarios without chemical coagulation that approaches energy neutrality, the higher relative independence from the electricity grid and offsets of CO_2 emissions may offer additional advantages of transforming an existing WWTP to a HiCS-B system. It should be noted that the presented CAPEX only includes the investment costs of the primary stage. Investment costs for secondary treatment, which were not included in this analysis, may constitute a substantial part of total WWTP costs, depending on the state of loan repayment. It was outside the scope of this work to estimate whether the addition of a primary treatment stage has an influence on the CAPEX costs of the secondary stage, considering that the daily COD load to the secondary stage will be lowered in

presence of a primary treatment process. *De novo* construction of a two-stage treatment plant might therefore require lower overall CAPEX investments compared with a stand-alone CAS plant retrofitted with a primary treatment stage.

4.4 Future prospects

With the development of shortcut nitrogen removal technologies, a larger fraction of COD will become available for recovery during primary treatment, and the COD recovery during primary settling, HiCAS or HiCS should be optimized accordingly. When comparing the HiCAS and HiCS scenarios with nitrogen removal by nit/denit, operational costs are near-identical (**Figure 5.3 B**). In that case, the choice between a HiCAS or HiCS system is primarily determined by the investment costs, which are 33% lower for a HiCS system than for a HiCAS system (primary settlers included), because of the 61% lower volume of the HiCS reactors.

Compared to the operational costs of a CAS system, switching to a two-stage system with HiCS as a primary treatment technology results in cost savings that are still relatively modest. In order to further decrease overall operational costs, additional optimization is needed along the wastewater treatment process. The sensitivity analysis showed that the overall plant OPEX is most sensitive to the operational parameters of the primary treatment stage (the % recovery of COD as sludge and the sludge digestibility), as well as the parameters that affect the cost of sludge disposal (the disposal cost itself, the COD/TSS and VSS/TSS ratios of the primary sludge, and the % solids capture during dewatering). Consequently, to lower operational costs of the wastewater treatment process, optimization efforts should primarily focus on increasing the production of primary sludge, and this has been the purpose of several recent studies on high-rate activated sludge systems using different process configurations (Chapter 4; Ding et al., 2015a; Faust et al., 2014a; Jimenez et al., 2015).

A second and concomitant approach should be to optimize the sludge digestibility and biogas production rates (De Vrieze et al., 2013; Jenicek et al., 2013). The anaerobic digestibility of the primary sludge was assumed to be 0.59 g $COD_{removed}$ g^{-1} COD_{fed}, which is a low estimate. Depending on the conditions, studies have found digestibility values of over 0.70 g $COD_{removed}$ g^{-1} COD_{fed} for high-rate sludge (Ge et al., 2013; Meerburg et al., 2015). If the digestibility of the HiCS sludge were increased to 0.7 g $COD_{removed}$ g^{-1} COD_{fed}, the scenarios with HiCS as primary treatment would all achieve energy neutrality without addition of chemical coagulants (net energy production of 1.7 to 1.8 kWh PE^{-1} y^{-1}). In that case, an OPEX value of € 2.23 to 2.28 PE^{-1} y^{-1} is obtained, and combined CAPEX + OPEX costs would be € 2.67 PE^{-1} y^{-1} for the HiCS-B N/DN scenario. This demonstrates that, given some optimization of sludge digestibility, the HiCS system allows net energy-neutral wastewater treatment at an overall operational + investment cost that is considerably more favorable than conventional treatment.

As a third approach, sludge disposal costs should be minimized, because these costs can take up to 65% of total gross operational costs in case chemical coagulants are added. A WWTP should therefore consider alternative routes of sludge disposal, such as on-site drying and incineration, or

explore novel and potentially self-repaying technologies such as supercritical oxidation (Griffith & Raymond, 2002; Svanström et al., 2004), pyrolysis and biochar formation (Fonts et al., 2012; Nansubuga et al., 2015).

Prior to full-scale application of two-stage wastewater treatment, a detailed case-specific study is needed to provide a more accurate estimate of the benefits of different primary treatment technologies. Perhaps less straightforward than constructing a *de novo* plant is the retrofitting of a two-stage system to an existing single-stage installation. Whereas this may be technologically feasible, care should be taken that no unforeseen drawbacks occur, e.g., when the secondary treatment basin becomes overdimensioned for its new purpose, or when equipment is abandoned that was not yet depreciated.

In conclusion, this study demonstrated that, using current technologies for nitrogen removal and anaerobic digestion, the HiCS system may achieve wastewater treatment at near-energy neutrality, and with operational costs lower than stand-alone CAS treatment or any other two-stage system included in the analysis. The amortized capital costs for converting an existing stand-alone CAS installation into a two-stage HiCS + B-stage system are lower than the simultaneous savings in operational costs, which makes a two-stage HiCS system more economically attractive than conventional wastewater treatment. Further optimization of COD recovery in the HiCS system may necessitate the development of nitrogen removal technologies in the secondary treatment stage with a COD demand lower than that of nitrification/denitrification. Given further optimization, the HiCS process may play an important role in the development of energy-neutral and more sustainable wastewater treatment.

5 Acknowledgements

F.A.M was supported as doctoral candidate (Aspirant) by the Research Foundation Flanders (FWO-Vlaanderen), and received additional support from the King Baudouin Foundation (Ernest du Bois prize). S.E.V was supported as postdoctoral fellow by FWO-Vlaanderen. N.B. was supported by the Inter-University Attraction Pole (IUAP) 'μ-manager' funded by the Belgian Science Policy Office (BELSPO, P7/25). We thank Bert Bundervoet and Marvin Zegers from Colsen NV for assistance with the CAPEX calculations, and Jo De Vrieze for feedback on the manuscript.

CHAPTER 6:

GENERAL DISCUSSION AND FURTHER PROSPECTS

1 State of progress

The purpose of this doctoral study was to provide a better understanding of the microbial ecology of high-rate activated sludge systems and develop operational strategies to maximize their potential for energy recovery from wastewater. To this purpose, the microbial ecology of a high-rate community was monitored over the course of a year, and compared to the low-rate community of an AB-system (**Chapter 2**). It was found that high-rate communities distinctly differ from low-rate communities in the sense that they have a lower richness and evenness, are more variable over time but less influenced by environmental factors. More research is needed to understand the link between community structure and functional output of HRAS systems. In **Chapter 3**, a comparison was made between HRAS systems with a high-rate conventional (HiCAS) and the novel high-rate contact stabilization (HiCS) configuration, to evaluate whether the feast-famine regime of the HiCS configuration has the potential to increase the biosorption capacity of the sludge. The HiCS process achieved a higher net recovery of COD than the HiCAS process. In subsequent reactor experiments with high-strength wastewater (**Chapter 4**), the COD recovery was optimized by varying the SRT, contact time and stabilization time of the HiCS process. Up to 55% of influent COD was recovered as sludge at an optimal SRT of 1.3 d, a contact time of 15 min and a stabilization time of 40 minutes. PHB measurements showed that intracellular storage was not a major pathway of COD removal, which was likely dominated by surface adsorption, settling and aerobic biomass growth. Further research should assess the performance of the HiCS process under field conditions of domestic wastewater treatment, such as lower influent strength and load fluctuations. The options for further optimization of the biosorption capacity and solid/liquid separation of the HiCS sludge should be further explored. The HiCS process should be compared with other primary treatment technologies, to assess the advantages and disadvantages of each technology in practice. **Chapter 5** presents an estimation of the net energy demand, OPEX and CAPEX costs of a full-scale two-stage system using different technologies for primary and secondary treatment. The scenarios with HiCS as primary treatment could achieve near-energy neutral wastewater treatment, and had the lowest operational costs of all scenarios considered, differing slightly across the secondary and side stream technologies used. Chemical flocculation could help to achieve energy positivity in all scenarios but drastically increased operational costs. Taking into consideration the capital investments for upgrading a stand-alone CAS installation to a two-stage system, the HiCS + B-stage system had a combined CAPEX + OPEX cost lower than that of the other primary treatment options (none, primary settling, or HiCAS) when N/DN was used for secondary nitrogen removal. This demonstrates that, in many cases, it may be economically favorable to retrofit a HiCS system into an existing wastewater treatment plant. The lower overall costs of the HiCS + B-stage system were primarily due to the lower volume requirements of the HiCS reactors compared to the HiCAS reactor, and the fact that no chemical addition was required. Further work should incorporate detailed economic aspects of other factors such as phosphorus removal, system control and maintenance, and assess the impact of optimization efforts for various sub-processes on the overall sustainability of wastewater treatment.

In this general discussion, additional aspects are addressed of understanding, optimizing and controlling high-rate activated sludge systems for energy recovery from wastewater. The potential role of ecological studies on sludge communities for a better management of the 'microbial resources' in HRAS systems will be discussed. The prospects of further technological optimization of COD recovery in the HiCS system will be explored. The importance and recent progress of model-based research for planning and design of the HiCS process is discussed thereafter. Finally, alternative valorization pathways, as opposed to energy production, of sludge from non-sewage wastewaters will be discussed, from the point of view that COD in wastewater can be considered a limited resource. The discussion ends with a general conclusion.

2 Microbial resource management of high-rate communities

A major aim of microbial ecologists is to understand how different community functions are performed and how they can be controlled (see Chapter 2). A wide range of bacteria is known to be involved in heterotrophic removal of COD (McIlroy et al., 2015), and heterotrophic removal itself comprises a range of different processes, such as surface adsorption and hydrolysis, oxidation of rapidly versus slowly degradable COD, storage, fermentation, denitrification, etc. Each of these processes may be dominated by one or more populations within the bacterial community. To determine which of the heterotrophic bacteria have a positive effect on desirable functions of activated sludge, such as adsorption or settling, and understand how these can be selectively favored, it is important to move beyond community ecology with a 'descriptive' approach, to an approach that incorporates functional analyses. A number of studies on 16S rRNA genes has described a clear correlation between the structure of an activated sludge community, and a function such as sludge bulking (Hesham et al., 2011), process stability (Gentile et al., 2007; Saikaly & Oerther, 2011), and overall functional richness (Johnson et al., 2014). However, correlation between microbial abundance and system functions do not always imply causation. In analogy of the famous postulates by doctor Robert Koch for determining a causal relationship between a micro-organism and a disease (Koch, 1882), the establishment of a causal abundance-function relationship in activated sludge communities may require rigorous investigation of the correlation in terms of strength, consistency and specificity, and even experimental manipulation (de los Reyes III, 2010). This is challenging, because activated sludge systems are open mixed cultures which rely on complex species-species interactions and contain numerous species that are not culturable in isolation.

For a more complete view on ecosystem functions, abundance-based 16S rRNA studies may be complemented by activity-based metagenomic studies on functional genes. Given the complexity of the heterotrophic metabolism, with many parallel pathways for the metabolization of various substrates into various products, it may be challenging to select which genes can be studied to ensure a sufficient degree of specificity and coverage for the process of interest (e.g., EPS production, substrate storage, oxidation, …). This means that the studied genes should only be involved in the

process of interest, not in other heterotrophic or autotrophic pathways, and should cover a broad span of parallel pathways if the process includes various possible substrates and products. For example, the production of EPS is of major importance for functions such as bioflocculation and settling, but EPS have a rich and variable composition, and the genetic pathways of EPS production are therefore complex. Studies that link EPS-associated genes with specific activated sludge bacteria and system functions are scarce (e.g., Albertsen et al., 2013; Wan et al., 2015; Zhang et al., 2015a). Other activated sludge functions, such as ammonia oxidation, are better understood in terms of which microorganisms and genes are involved in the process, and functional studies can be adequately carried out on genes and their expression using quantitative real-time PCR (qPCR) with a limited set of primers (Dionisi et al., 2002; Rotthauwe et al., 1997; Tsushima et al., 2007).

Whole metagenomic or metatranscriptomic analyses can provide insight in the metabolic capacity of an activated sludge community, by matching the detected sequences to databases of genes with a known function. Subsequently, correlation analysis with environmental and operational variables can give a better understanding of the influence of external factors on the functional potential of the microbial community. In a metagenomic (i.e., presence-based) approach, a study on functional genes can explore the metabolic potential of a sludge community, and the presence of functional genes can be correlated to the presence of certain bacterial species, to the colonization of micro-organisms from the influent, to environmental and operational variables, and to the COD and nutrient removal performance of the plant, in order to establish a fundamental understanding of how the metabolic potential of the community is influenced. In a metatranscriptomic (i.e., activity-based) approach, a study on functional genes can describe the actual metabolic activity of the community, and correlation analyses can reveal a more direct and mechanistic relationship between environmental and operational factors, the metabolic activity of the microbiome, and plant performance. This may help develop operational strategies that aim to directly modify the microbial community of an activated sludge system in order to control its performance. The establishment of robust correlations between functional genes and plant performance may lead to the development of quantitative assays that target genetic markers in the sludge metagenome or metatranscriptome, which could function as a diagnostic tool of the metabolic state of the sludge system. Finally, a comparison between the metagenome and the metatranscriptome can provide insight in the role of ‘seed bank’ species and the mechanisms of their reactivation. Attempts have been made to deduct the functional or metabolic potential of a community from 16S rRNA genes (Aßhauer et al., 2015; Gonzalez-Martinez et al., 2016; Ju & Zhang, 2014), although it can be argued that a comprehensive view of the metabolic potential of a community always requires sequencing of the whole metagenome or metatranscriptome, rather than only targeting the 16S rRNA.

It is still a long way to transition from ‘functional community analysis’ (i.e., a descriptive analysis of the connection between microbial community structure and function) to ‘microbial resource management’ (MRM; i.e., the development of technological tools to provide a direct control over the microbial community members and their functional performance). Functional community analysis is still an emerging research field, and much more progress is needed before the development of

microbial resource management tools will become possible. Furthermore, compared to the complexity and richness of the activated sludge ecosystem, the current set of operational control tools available to wastewater treatment plants are limited. A wide array of subtle yet reliable control tools needs to be developed in order to achieve process optimization at an MRM level. Hanemaaijer et al. (2015) argue that, in order to gain rational and interventional control over microbial communities, it is necessary to integrate genomic, physiological and stoichiometric knowledge of the system in a modeling approach on two levels. The first level would be genome-based modeling, in which potential metabolic fluxes are predicted from the genomes of single species, and which has made much progress in recent years (for example, Cuevas et al., 2016; Yurkovich & Palsson, 2016; and references therein). The second level would be metagenome-based modeling, in which community-scale stoichiometric models are built by combining genome-based predicted metabolic fluxes of single species with species abundance data and measurements of the community-level fluxes. Community-scale modeling will play an essential role in providing a mechanistic understanding of the structure and function of microbial communities, but because of its computational complexity, its reliance on exhaustive physiological databases, and the need for extensive analytical and experimental data, it has not been fully developed yet (Hanemaaijer et al., 2015; Röling & van Bodegom, 2014).

3 Prospects for further technological optimization

Parallel to the efforts to develop community-oriented MRM tools (see section 2), the development of process-oriented operational control tools will remain of major importance for wastewater treatment, and is arguably the more 'pragmatic' route to achieve technological progress. With respect to the HiCS system, the COD recovery performance should be further optimized.

To improve recovery of COD by the HiCS process, technological strategies should be developed that favor non-destructive removal pathways (i.e., cell growth and biosorption, which includes storage, accumulation and surface adsorption of soluble and particulate COD) over destructive pathways (i.e., oxidation). The HiCS process is able to achieve similar rates of COD recovery compared to conventional high-rate configurations, but with very low percentages of COD oxidation (see Chapters 3, 4 and 5). As a result, relatively high percentages of COD remain in the primary effluent. Even with the SRT, contact time and stabilization time optimized for COD recovery (see Chapter 4), the HiCS system still has a washout from 31% (high-strength wastewater, see Chapter 4) to 50% (medium-strength wastewater, see Chapter 5) of incoming COD. As was shown in Chapter 5, an ideal primary treatment technology has a high recovery and low oxidation of COD, while sending a sufficient amount of COD to the primary effluent to complete the nitrification/denitrification cycle during secondary treatment. Soluble COD is a more direct substrate for denitrification, because it does not need to be hydrolyzed. Therefore, instead of optimizing all biosorption pathways at once, it is proposed that optimization of COD recovery in the HiCS system should primarily focus on

bioflocculation (i.e., floc formation and surface adsorption of substrate COD_{part} and COD_{coll}), and (2) solid/liquid separation.

3.1 Optimizing the contactor and stabilizer

A number of parameters affecting bioflocculation may be considered for optimization. A first parameter is **shear**, because while increased mixing and aeration may enhance the performance of biological processes, too high shear forces may interfere with the bioflocculation process (Haugaard Mikkelsen & Keiding, 1999). Controlled, elevated shear conditions may act as a selection pressure to promote formation of adhesive EPS and produce flocs that are more resistant to erosion (Menniti et al., 2009). Shear forces are positively correlated with the density, hydrophobicity and EPS production of sludge flocs (Tay et al., 2001), but the effect of shear may be less pronounced at higher substrate loading rates (Tay et al., 2003), as would be the case in high-rate systems. A study on a high-rate pilot installation treating low-strength influent compared a HiCS configuration with a conventional HiCAS configuration under similar shear conditions in the contact tank (i.e., prior to settling), and found that the HiCS system achieved a higher COD recovery and lower washout through the effluent (Rahman et al., 2016). The pilot study indicated that the shear force prior to settling could not have played a role in the better performance of the HiCS system. However, the HiCS reactor did experience a higher shear force in the stabilization tank, and direct comparison between the HiCS and HiCAS systems was difficult because of differences in SRT. High shear forces can cause sludge to deflocculate, and reflocculation occurs when the shear force is lowered (Nopens, 2005). This raises the possibility of using shear-induced deflocculation of sludge flocs during or after the stabilization phase, to improve the attachment of particulate substrate during reflocculation in the contact phase. It is possible that optimization efforts of the shear forces in the HiCS system may indicate that the highest bioflocculation capacity is obtained under changing shear conditions, with the highest shear occurring during the stabilization phase before influent contact, and the lowest shear occurring during the contact phase before settling. However, the potential benefits of such a deflocculation-reflocculation cycle in the HiCS system remains to be investigated.

A second parameter to optimize is **dissolved oxygen** (DO). In terms of oxygen concentration, the stabilization phase should maintain a DO level well above the half-saturation coefficient of 0.2 mg L^{-1} for heterotrophic growth (Henze et al., 2000), to ensure that oxygen is not a limiting factor. Conversely, the oxygen concentration during the contact phase should be kept as low as possible, so as to limit aerobic COD degradation and maintain a high selective pressure towards sorption and storage of substrates. On the other hand, short-term exposure to low DO conditions may lead to sludge deflocculation and the deterioration of particle settleability (Zhang & Allen, 2008), which has been attributed to the inhibition of EPS production at low DO concentrations (Starkey & Karr, 1984), the inhibition of aerobic metabolism (Wilén et al., 2000) and microbial reduction of Fe(III), an ion with a strength-enhancing effect on flocs because of the formation of cation bridges (Caccavo et al., 1996; Wilén et al., 2000). However, in the above studies, deflocculation during anaerobic conditions occurred in the order of hours, and the studies were performed with sludge grown in aerobic conditions at long SRT. Activated sludge processes with short anaerobic phases alternated with

aerobic phases, such as the processes designed to select for biological phosphorus removal (see Chapter 1), generally do not suffer from settleability problems (Schuler & Jang, 2007), and in the case of selector systems, a feast-famine type alternation between anaerobic and aerobic phases is even used to stimulate floc formation (Chudoba et al., 1973). The deflocculating effect of low DO concentrations is more pronounced at a high SLR (0.8 g BOD_5 g^{-1} VSS d^{-1}) compared to a low SLR (0.08 g BOD_5 g^{-1} VSS d^{-1}) (Starkey & Karr, 1984). For these reasons, it is unclear whether the HiCS system, where sludge is acclimated to fast alternations between low DO and high DO conditions, the SRT is low and the SLR high, experiences deflocculation effects during the anaerobic conditions of the contact phase. Future work should investigate whether the avoidance of complete anaerobic conditions during the contact phase may lead to improved bioflocculation in the HiCS process. However, when oxygen is supplied in the contact phase, unwanted side-effects may occur, such as a higher fraction of COD oxidation and increased shear. The sensitivity of the bioflocculation and COD recovery performance to DO levels in the contact phase will ultimately have practical implications for the HiCS system, in the sense that it may (or may not) be advisable to achieve mixing of the MLSS during the contact phase by means of aeration, as opposed to mechanical mixing.

Although addressed in Chapter 4, contact and stabilization times may need continuous optimization, depending on the wastewater conditions. To achieve an effective COD removal, the **contact time** needs to be long enough to allow completion of the adsorption, storage and flocculation process, but otherwise as short as possible, to avoid excessive operational costs. Depending on the influent composition and strength relative to the available biomass in the reactor, the optimal contact time may differ. For a given type of influent, an indication of optimal contact time can be obtained in a biosorption batch experiment, where the decrease in supernatant COD concentration is monitored in function of contact time, and an optimal contact time can be selected as the earliest time at which a stable (or minimum) supernatant COD concentration is reached. To achieve an effective feast/famine regime, the **stabilization time** needs to last long enough to allow the sludge to reach a near-endogenous state. Only after degradation of the adsorbed and stored COD during famine conditions does the sludge regain its full substrate uptake capacity during feast conditions (Cech & Chudoba, 1983). Experiments at different stabilization times indicated that a minimal t_s of 30 to 40 minutes is required for medium to high-strength wastewater (Chapters 3 and 4, Huang & Li, 2000). However, the required stabilization time may differ with influent strength or SLR. Respirometry can be a powerful tool to monitor the progression from exogenous to endogenous respiration (see Chapter 3). For a given influent and SLR, an indication of optimal stabilization time can be obtained in a respirometric batch experiment, where an optimal stabilization time can be selected as the earliest time at which a near-endogenous respiration rate is reached. By combining respirometric data (i.e., the oxygen consumption over time) with substrate removal data and titrimetric data (i.e., the uptake-associated pH change of the bulk liquid over time), the relative contribution of storage and sorption, cell growth and oxidation can be determined (Dircks et al., 1999; Gernaey et al., 2002; Karahan-Gül et al., 2002). Potentially, respirometric measurements may allow developing a control

strategy in which the stabilization time is controlled based on the progress of the oxygen uptake curve.

Additional parameters, such as pH and SLR, may impact HiCS performance, but are often a result of pre-fixed conditions such as wastewater characteristics, flow rates and SRT, and less practical to be optimized in a controllable manner.

3.2 Optimizing the solid/liquid separation

A critical step in the recovery of COD from wastewater is the separation of (bio)flocculated material from the bulk liquid. Gravitational settling is the most widespread and cost-effective method for solid-liquid separation, and was also used during the HiCS experiments in this work (see Chapters 3, 4 and 5). However, systems with a high SLR often suffer from poor sludge settleability (Chao & Keinath, 1979; Modin et al., 2014), and optimization of the solid/liquid separation is therefore necessary. With regards to predicting and improving the gravitational settling performance of the HiCS system, a profound knowledge of the settling behavior of the sludge is critical.

3.2.1 Separation technologies

A first approach to improve the solid/liquid separation is to adopt technologies other than gravitational settling. High-rate systems have been operated in combination with membrane filtration (Akanyeti et al., 2010; Faust et al., 2014b; Hernandez Leal et al., 2010), dynamic filtration (Roest et al., 2012), and dissolved air flotation (Ding et al., 2015a). In many cases, physical technologies, including gravitational settling, can be made more efficient by dosing chemical coagulants and flocculants (Metcalf & Eddy, 2003). While exploratory batch experiments have quantified the increase in COD recovery due to coagulant addition in the HiCS system (see Chapter 5), further work should address all aspects of coagulant and flocculant addition on system performance, in function of the dosage ratio. Possible effects include an improved effluent quality, improved phosphorus removal, decreased sludge digestibility, increased operational costs, and decreased bioflocculation capacity due to the presence of precipitated coagulants in the return sludge.

A second approach of optimizing the solid/liquid separation is to improve the bioflocculation capacity and settleability of the sludge by means of biological selection. Optimization of the bioflocculation capacity has been discussed in Section 1.2. However, a selection pressure to favor floc formation does not necessarily lead to improved settleability. Sludge settleability not only depends on the formation of flocs or the absence of extensive filamentous growth, but also on floc shape and density. There are indications that selection for dense sludge flocs with a low porosity can be achieved by avoiding diffusion-limited gradients of substrate and oxygen inside the sludge flocs (Martins et al., 2003a; Martins et al., 2011). This could be partially achieved by means of pulse feeding and pulse aeration. Selection for dense flocs, and even granules, can be achieved by a combination of a feast-famine regime with pulse feeding at a high volumetric loading rate, high

hydrodynamic shear forces, air-lift column configuration of the reactor, and a relatively short settling time (Beun et al., 1999; Liu et al., 2005).

3.2.2 The settling behavior of HiCS sludge

As sludge settles, the solids concentration increases and the settling dynamics change. Settling can be described according to four different mechanisms, in increasing order of solids concentration and decreasing order of settling velocity (Ekama et al., 1997): (I) Stokesian settling, where particles settle individually with a velocity determined by their size and density, according to Stokes' law, and are dispersed through the water matrix during settling, (II) zone-settling or hindered settling, where particles are in close enough proximity that they settle as a single sludge blanket with uniform velocity, and a clear interface between the sludge mass and the supernatant exists, and (III) compression settling, where the settled sludge particles are in direct physical contact and can only be further compacted by gravitational force. Stokesian settling can be subdivided into (Ia) discrete settling, where particles are present in such low concentrations that they do not interact with one another, and (Ib) flocculent settling, where flocculation occurs as particles collide, and settling velocity increases with floc size. There can be an abrupt transition between these settling mechanisms with increasing solids concentration (Mancell-Egala et al., 2012; Mancell-Egala et al., 2016). The solids concentration at which discrete settling changes to flocculent setting is called the threshold of flocculation (TOF). The concentration at which flocculent settling changes to hindered settling is called the limit of Stokesian settling (LOSS). The transition from hindered settling to compression settling occurs when a critical solids concentration (X_{crit}) is reached, after which only compression takes place.

As sludge concentrations typically change with location inside a settler (Torfs, 2015), the settler performance is dependent on different settling mechanisms. For example, discrete settling in the top layer of the water column influences the effluent TSS concentration. The hindered settling velocity determines the height of the sludge blanket and the risk of sludge washout when the sludge blanket is too high. Compression settling influences the concentration of the return sludge. A low TOF value is desirable, because flocculation and thus faster settling will occur even at low sludge concentrations. A high LOSS value is desirable, because flocculent settling will occur even at high sludge concentrations and prevent the formation of a high sludge blanket that may wash out to the effluent (Mancell-Egala et al., 2016).

The settling propensity of sludge can be monitored throughout the range of different settling mechanisms. In the lower concentration range, where discrete settling occurs, sludge particles can be fractionated according to their settling velocity. These fractions are called initial settling classes (ISC), and can be determined in a batch experiment by measuring the fraction of TSS that remains in the supernatant layer with a known height after different settling times, for conditions where the sludge concentration is below the TOF. In a pilot study, the ISC were determined for a HiCS process with an SRT of 1.7 d, and compared to the ISC of a HiCAS configuration with an SRT of 0.4 d. Operational details of the pilot study are described by (Rahman et al., 2016). The ISC fractionation is

shown in **Figure 6.1**. Of the two systems, the HiCS sludge had a better settling performance during discrete settling, as indicated by the larger fractions of non-flocculated particles with a high settling velocity. As these differences manifested below TOF concentrations (i.e., during discrete settling), the better settling performance of the HiCS sludge indicated that the non-flocculated sludge particles likely had higher densities or larger diameters compared to the HiCAS sludge. However, it should be noted that direct comparison between the two systems is difficult because of the difference in SRT, and that the HiCAS system suffered from sludge washout during these measurements. Therefore, the HiCS sludge may not possess better discrete settling characteristics compared to HiCAS sludge in all situations.

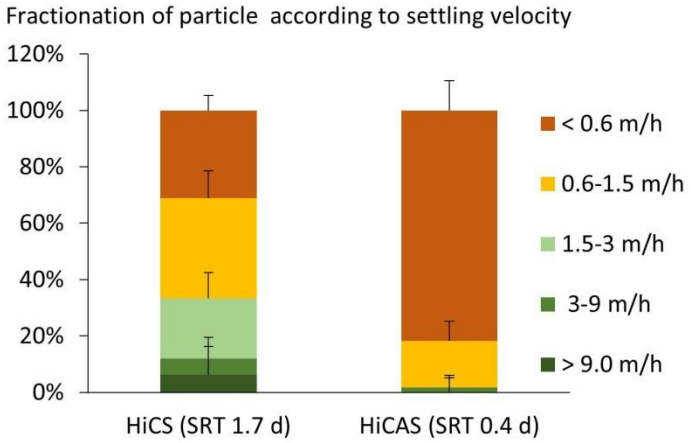


Figure 6.1: Initial settling classes of sludge from a pilot plant, comparing a HiCS configuration with a HiCAS configuration (own work, described by Rahman et al., 2016).

In the same pilot study, the TOF was determined from batch experiments in which sludge, at different concentrations, was allowed to settle and the supernatant was collected down to a height that corresponded to a settling velocity of 1.5 m h^{-1}. The TOF was the solids concentration after which a sudden drop in supernatant TSS was observed, due to the start of flocculent settling. It was found that the TOF of the HiCS sludge was 156 ± 32 mg L^{-1}, while the TOF of the HiCAS system, which suffered from sludge washout, could not yet be reached at concentrations above 1300 mg L^{-1}. This indicates that a HiCS configuration may, in certain cases, result in a better flocculation affinity compared to the HiCAS system. Moreover, these results suggest that the washout of sludge is, in some cases, associated with a decrease in flocculation affinity, and that the TOF may be a valuable indicator of the settling behavior of sludge.

In a different series of experiments, the settling behavior of HiCS sludge was compared to that of CAS sludge during hindered settling. To this purpose, a settling batch test was performed to record the height of the sludge blanket over time, where the settling velocity was determined from the slope of the linear part of the curve. The results of the hindered settling batch tests are shown in **Figure 6.2**.

Over a wide range of concentrations, the HiCS sludge maintained higher settling velocities compared to CAS sludge. Moreover, the results indicated that the critical concentration at which compression settling commences (X_{crit}) was well above 8.5 g L^{-1} for the HiCS sludge, because across all concentrations, the settling curves showed an initial linear descent. Conversely, the CAS sludge had a X_{crit} of 5.5 g L^{-1}, as indicated by the absence of an initial linear descent above this concentration (Torfs, 2015). The higher settling velocity during hindered settling and higher X_{crit} of the HiCS system suggest that HiCS sludge is able to form more compact sludge blankets and may be less likely to suffer from sludge washout compared to CAS sludge.

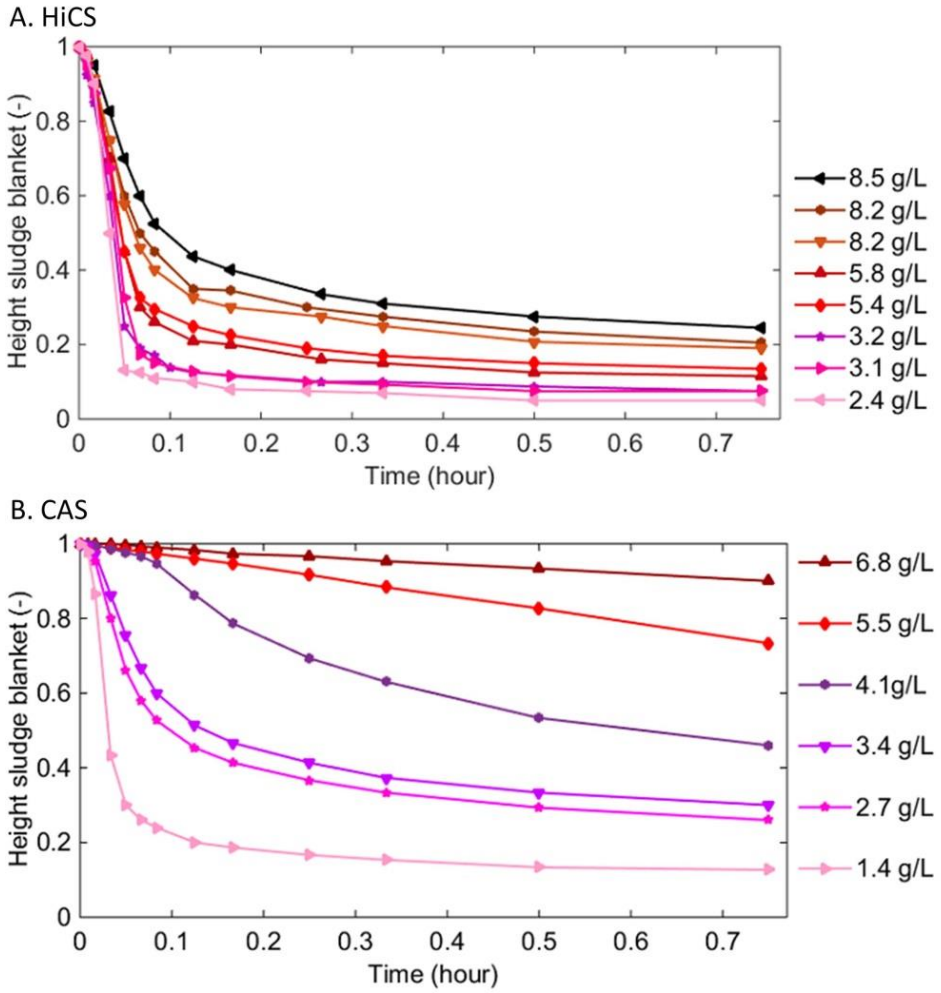


Figure 6.2: Sludge blanket height in function of time during hindered settling for different sludge concentrations. (A) HiCS sludge, (B) CAS sludge from the Destelbergen WWTP, Belgium. Adapted from De Smedt (2015) and Torfs (2015).

The Takács equation describes the sludge settling velocity in function of the solids concentration (Takács et al., 1991):

$$v_{hs} = v_0 e^{-r_h(X-X_{min})} - v_0 e^{-r_p(X-X_{min})} \qquad \text{Equation 6.1}$$

with the additional condition that $0 \leq v_{hs} \leq v_{0,max}$. In this equation, v_{hs} is the settling velocity during hindered settling, v_0 is the maximum settling velocity, r_h is a constant characteristic for hindered settling, r_p is a constant characteristic for settling at low sludge concentrations, X is the solids concentration, X_{min} is the minimum attainable solids concentration at which no further settling occurs, and $v_{0,max}$ is the maximum settling velocity. The initial setting velocity for both types of sludge was plotted against the biomass concentration, as shown in **Figure 6.3**, and the Takács parameters were estimated.

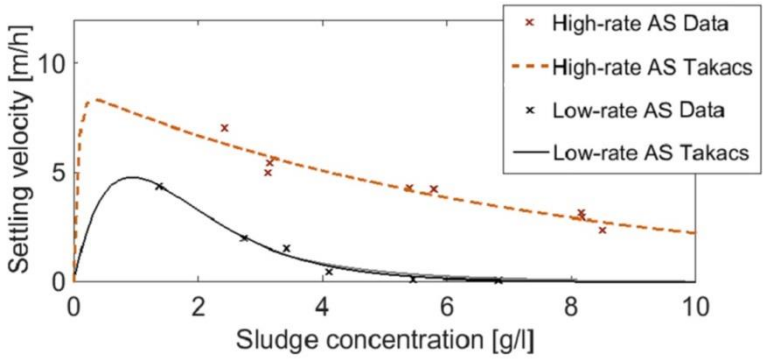


Figure 6.3: Settling velocity in function of sludge concentration, for HiCS sludge (High-rate AS) and CAS sludge from the Destelbergen WWTP, Belgium (Low-rate AS), along with fitted curves using the estimated Takács parameters. Adapted from De Smedt (2015).

Whereas not all of the Takács parameters could be accurately estimated from the data presented in **Figure 6.2**, the r_h was estimated to be 0.138 L g^{-1} for the HiCS sludge and 0.999 L g^{-1} for the CAS sludge (De Smedt, 2015). The lower r_h of the HiCS sludge indicates that the settling velocity during hindered settling diminishes less quickly with increasing sludge concentration, compared to the CAS sludge. It should be noted that settling batch tests only describe the behavior of the *settleable* fraction of sludge. During these experiments, the solids concentration of the supernatant of the HiCS system was considerably higher than that of the CAS system, indicating that the HiCS sludge had a higher non-settleable fraction.

All of the above results suggest that the settling behavior of HiCS sludge differs from that of HiCAS and CAS sludge, and may be better under certain conditions. Further study should perform a systematic comparison of the four different settling mechanisms between HiCS and HiCAS configurations under controlled conditions, and explore how the settling behavior can be further improved in order to optimize the overall solid/liquid separation.

4 Modeling the HRAS process

Mathematical modeling of the activated sludge process can be used to describe how the system behaves, given a set of pre-defined conditions. An accurate model is able to predict the performance of a system much faster than experiments can, and for a multitude of conditions. As such, accurate models can play an important role in the design and optimization of (novel) activated sludge processes. In general, a model calculates a set of state variables (e.g., effluent COD and nutrient concentrations, solids concentrations, removal efficiencies) from a set of input variables (e.g., influent concentrations, flow rates), using a set of expressions that describe the different conversion processes by means of model parameters (e.g., reaction rates, affinity constants, conversion ratios). The accuracy of a model depends on the biological or mechanistic plausibility of the model expressions and the accuracy of the parameter estimates. In order to develop a model that accurately describes the HiCS process, it is necessary to expand existing models to more accurately describe the behavior of different sub-processes such as adsorption, storage and oxidation. Experimental measurements need to be made to provide reliable estimates of the model parameters.

4.1 Expanding the ASM models

In the past decades, task groups of the International Water Association (IWA) have created mathematical models to describe the activated sludge process. These activated sludge models (ASMs) have been modified to different versions and expansions, to accommodate specific reactions and conversions. ASM1 models COD and nitrogen removal, and forms the basis of several model expansions. ASM2 and ASM2d expand the basis model with enhanced biological phosphorus removal. In ASM3, the basis model is restructured to include a more biologically plausible description of the hydrolysis, storage, growth and decay processes (Henze et al., 2000).

The ASM models are not particularly suited to model high-rate activated sludge processes. In high-rate systems, processes such as adsorption can no longer be considered instantaneous and need to be modeled separately (Larrea et al., 2002; Makinia et al., 2006). Because of their short HRT and SRT, high-rate sludge systems are not always able to remove all of the substrate that is theoretically 'biodegradable' (Haider et al., 2003). Therefore, when HRAS systems are modeled, the soluble biodegradable COD (S_B) may be divided into fast and slowly biodegradable fractions (S_{Bf} and S_{Bs}), which can be modeled with different maximum removal rates or affinity constants to create a preferential, fast removal of S_{Bf} and a slower removal of S_{Bs}.

Nogaj et al. (2015) proposed a modification of the ASM1 model to be suitable for high-rate activated sludge processes. They proposed two ways by which the differential removal of S_{Bf} and S_{Bs} could be modeled: in the dual substrate approach, S_{Bf} and S_{Bs} are removed simultaneously, but S_{Bf} has a higher maximum specific substrate uptake rate and is, this removed faster. In the diauxic approach, S_{Bf} is removed first, and its presence prevents removal of S_{Bs} through an inhibition term in the Monod equation, so that S_{Bf} removal only starts when S_{Bf} is nearly completely consumed. It is unclear which

of the approaches is more widely applicable to various conditions, but the dual substrate model was able to describe the experimental results of an HRAS pilot plant more accurately than the diauxic model (Nogaj et al., 2015). In either approach, removal of S_{Bf} and S_{Bs} by heterotrophs occurs along four pathways: oxidation to CO_2, transformation to cell biomass, or production of EPS (X_{EPS}) and storage polymers (X_{sto}). Inert and biodegradable colloids (C_U and C_B, respectively) are bioflocculated into the solids fractions (X_U and X_B), and this bioflocculation rate depends on the biomass and EPS concentrations. Conversions between the model variables are modeled by means of conversion coefficients, yield factors, maximum rate constants and half-saturation coefficients, similar to the other ASM models. The mass transfer between the variables of this model is schematized in **Figure 6.4**.

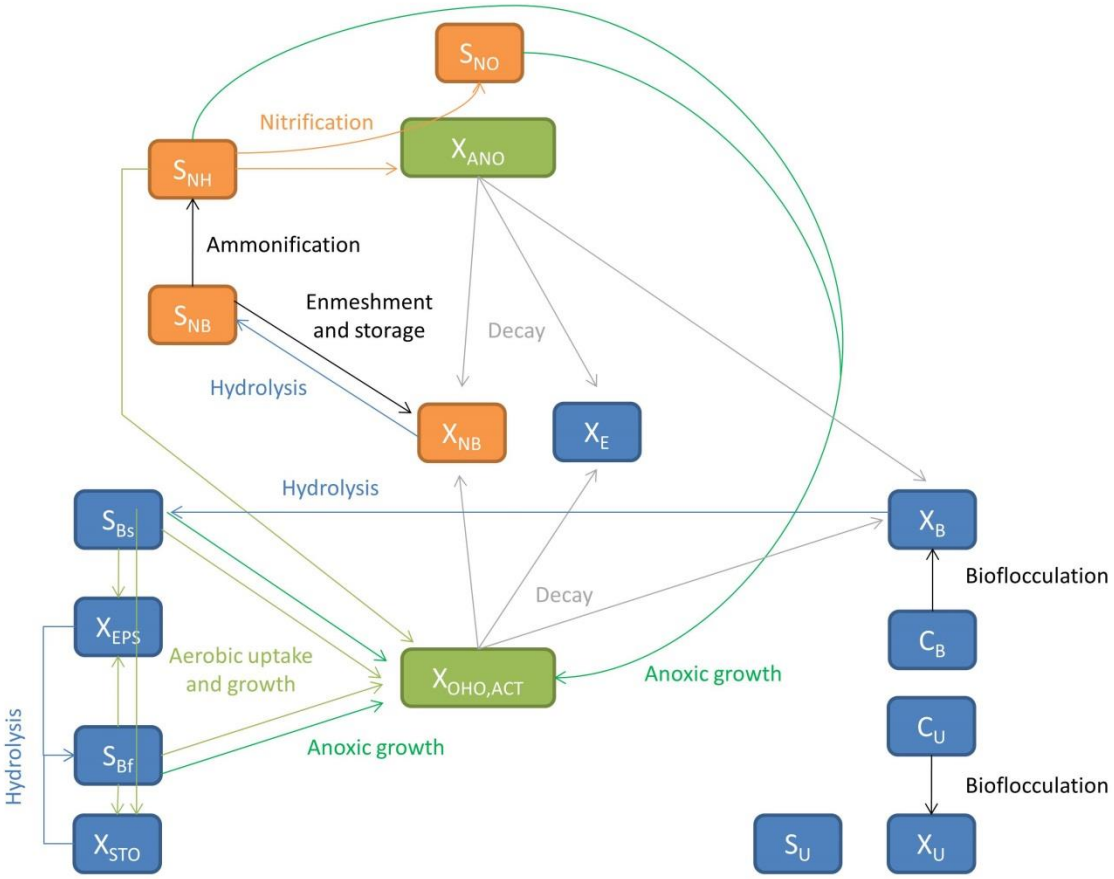


Figure 6.4: Mass flow scheme of state variables according to the HRAS model of Nogaj et al. (2015). Symbols are as defined in the Notation index and List of sub- and superscripts. Figure redrafted after De Smedt (2015).

In order to model the HiCS process, it is necessary to use a HRAS model that can accurately describe the processes of adsorption and storage, and which is able to separately model the processes in the

contact and stabilization phases. This can be achieved by using a generic HRAS model, and simultaneously running the models for the contactor and stabilizer, where the inflow of the contactor consists of the influent and the return flow from the stabilizer, and the inflow of the stabilizer consists of the settler return flow. However, a detailed calibration of the model parameters will be necessary before the model can accurately describe a HiCS system (De Smedt, 2015). The presence of a feast-famine regime may lead to substantial differences compared to conventional HRAS systems for parameters such as adsorption rate constant, and the proportionality constants for storage and EPS production.

4.2 Calibration of model parameters

The HRAS model proposed by Nogaj et al. (2015) is a relatively complex model, with more than 10 additional parameters on top of the 19 parameters already incorporated in ASM1. In order to calibrate model parameters, experimental data can be compared to a model output and the parameters manipulated until the output fits the experimental data reasonably well. Complex models often incorporate parameters that are difficult to measure with existing laboratory techniques. Moreover,, in complex models, there is a high chance that many parameters are correlated, which creates an identifiability problem because parameter calibration will be difficult to achieve in certain conditions. The problem of correlation and identifiability in relation to experimental conditions can be illustrated by the Monod expression (see Chapter 1):

$$\frac{dX}{dt} = \left(\mu_{max} \times \frac{S}{K_S+S} - b\right) \times X \qquad \text{Equation 6.1}$$

Depending on the substrate concentration (S), the Monod expression will approach different forms. When S << K_S, the expression will approach $\frac{dX}{dt} \cong \left(\mu_{max} \times \frac{S}{K_S} - b\right) \times X$, and the two constants μ_{max} and K_S are expressed as a ratio, $\frac{\mu_{max}}{K_S}$. This will make it impossible to separately identify them by fitting the model on experimental data, because when μ_{max} is increased, the model will fit the data equally well, as long as K_S is also increased. When S >> K_S, the expression will approach $\frac{dX}{dt} \cong (\mu_{max} - b) \times X$, and the value of μ_{max} can be more easily determined. Because the value of K_S is not known a priori, batch tests are often designed at a given ratio of substrate to biomass (S/X) instead. The behavior of the Monod expression at different S concentrations is one of the reasons why it was found that the identifiability of kinetic parameters from batch experiment data changes with different S/X ratios (Grady et al., 1996). The decay rate b does not suffer from the same identifiability problem, since it forms a separate term in the expression and, thus, has a stronger degree of independence from other parameters.

To estimate K_S and μ_{max} for high-rate sludge from an A-stage system (Breda WWTP, the Netherlands), Decubber (2014) used experimental data from respirometric batch tests to fit a simplified growth model, based on ASM1, considering only heterotrophic growth and decay . The identifiability of the two parameters was examined by calculating the model fit over a wide range of parameter values. For a batch experiment with a low S/X ratio, the parameters were highly correlated, resulting in

equally good model fits over a wide range of μ_{max} and K_S combinations (**Figure 6.5 A**). At a high S/X ratio, good model fits were only obtained within a small range of parameter values, indicating that in these conditions, the parameters μ_{max} and K_S can be estimated with high precision from a respirometric batch test (**Figure 6.5 B**). In order to keep parameter identifiability high but avoid that the fastest growing microorganisms dominate the experiment's outcome at too high S concentrations, it is suggested that batch experiments for the determination of μ_{max} and K_S be performed under conditions where $S > K_S$ (Decubber, 2014) and $S/X < 1/40$ (Grady et al., 1996).

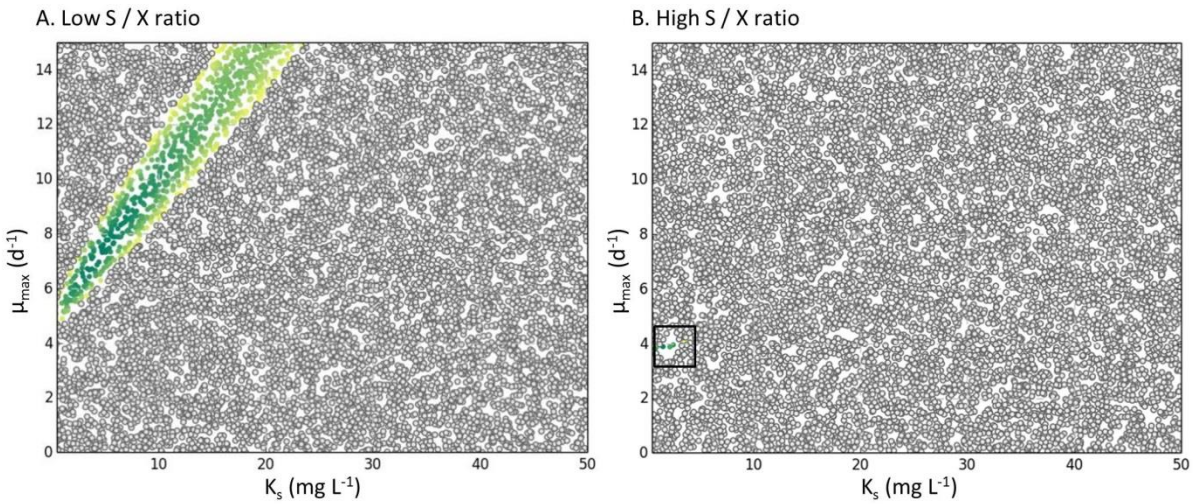


Figure 6.5: Results of Monte Carlo simulation with 10 000 shots for the parameters μ_{max} and K_S in a simplified ASM1 model describing the results of a substrate consumption experiment on high-rate activated sludge. (A) High S/X ratio; (B) low S/X ratio. Model fits are color-coded from grey (bad fit) to green (good fit), calculated as the sum of squared errors (SSE). Figure adapted from Decubber (2014).

De Smedt (2015) adapted the HRAS model proposed by Nogaj et al. (2015), to apply it to a HiCS system operating on high-strength wastewater, using the diauxic approach of S_{Bf} and S_{Bf} removal. A global sensitivity analysis was performed on this model, by evaluating the relative influence of perturbations of the parameters on the predicted state variables. The identifiability of a parameter manifested itself when perturbation of that parameter caused a relatively large change in the outcome of one of the state variables. In other words, for parameters that had no strong influence on any state variable, it would likely be difficult to obtain a parameter estimation by fitting the model to experimental data. Moreover, a simplification of the model structure could be considered by leaving out those parameters which did not influence the predicted state variables in any circumstances. This would lower computation times, but could also impact the biological plausibility of the model and limit the conditions in which it is applicable. It was found that, for a HiCS system, the parameters with the least influence on model outcome were the half-saturation coefficients for hydrolysis of organics entrapped by bioflocculation ($K_{b,hyd}$), hydrolysis of storage products ($K_{sto,hyd}$), and EPS-mediated bioflocculation (K_{EPS}) (De Smedt, 2015). This could be explained by the high

substrate concentrations in the influent of the HiCS system, which caused the respective Monod reactions to occur well above the half-saturation point.

Subsequently, reactor and effluent COD measurements (fractionated into COD_{part}, COD_{coll} and COD_{diss}) of a high-strength HiCS reactor experiment were fitted to the model outcome while performing perturbations of the parameters, to determine the potential identifiability of parameters based on data that is routinely collected during reactor experiments (De Smedt, 2015). Ten parameters were found to be potentially identifiable, although in practice, the actual identifiability (i.e., the precision of the estimate) might still suffer from correlations between parameters under certain conditions, as shown in **Figure 6.5**. Parameters that could be estimated from the experimental COD measurements were the yield for aerobic heterotrophic growth ($Y_{OHO,aer}$), the maximum heterotrophic growth rate on slowly biodegradable substrate ($\mu_{OHO,slow}$) and the heterotrophic decay rate (b_{OHO}). The other model parameters either were correlated and required different experimental conditions, or required measurement of additional variables such as the fractionation of the heterotrophic growth yield into cell growth, EPS formation and storage.

These first efforts to model the HiCS system demonstrated that it is important to review the structure of existing ASM or HRAS models to incorporate important biological processes, while avoiding excessive model complexity. Parameter identifiability can be assessed through global sensitivity analyses and by fitting model outcomes to experimental data. For a precise determination of model parameters, experimental measurements are needed over a range of different conditions.

5 Beyond energy recovery

Throughout this work, high-rate activated sludge processes have been studied with the main goal to use the recovered COD for energy production through anaerobic digestion. Anaerobic digestion (AD) of sludge is the most widespread and technologically characterized method of recovering organic energy from wastewater. For municipal wastewater (sewage), anaerobic digestion has the advantage that its valued product, methane, can be recovered in relatively pure form from a contaminated source. However, for wastewaters free of pathogens and with a relatively simple composition, such as industrial wastewaters, other recovery pathways may be possible that produce more valuable end products.

The ‘carboxylate platform’ was proposed as a collection of biological and chemical pathways to produce short-chain carboxylates from feedstocks such as industrial and agricultural wastes (Agler et al., 2011; Angenent et al., 2004). Central to the carboxylate platform are the processes of hydrolysis and fermentation, performed by a mixed anaerobic culture. Since this is identical to the conversion processes of AD, apart from the last step (methanogenesis), fermentative production of carboxylates can be obtained by inhibiting methanogenesis during AD (Agler et al., 2011). Many industrial and agricultural waste streams contain high enough COD concentrations to allow bioproduct formation

through direct fermentation without the need of a pre-concentration step such as HRAS treatment, such as thin stillage (Andersen et al., 2015; Gonzalez et al., 2010). However, for other types of wastewaters, pre-concentration of the COD is necessary before recovery of carboxylates can be economically attractive. As discussed in Chapter 1, high-rate activated sludge is a preferred technology to achieve pre-concentration of wastewater organics. As reviewed by Lee et al. (2014), a number of studies have been performed on the production of carboxylates from primary and secondary sludge. Even for municipal wastewater, fermentation of high-rate sludge has been explored as a valuable alternative to AD for valorization of the recovered COD (Cagnetta et al., 2016).

Microbial proteins are another high-end product that can be obtained from COD recovered from wastewater. Microbial proteins, or single cell proteins (SCP) may be used as animal fodder or human food product, as long as the COD source is pathogen- and contaminant-free. Like all proteins, SCP contain a high amount of nitrogen. Therefore, apart from recovering COD, SCP production from waste streams has the additional advantage that waste nitrogen is also recovered. The up-cycling of COD and nitrogen from a waste product directly into a high-end food product makes SCP production an attractive technology in the transition to a more sustainable nutritional cycle. As reviewed by Anupama and Ravindra (2000), SCP have traditionally been produced by various micro-organisms, such as bacteria, fungi and algae, from a variety of waste substrates. While there is a widespread tradition to use the biomass of autotrophic organisms such as algae as a food source, these can mainly be used to recover nitrogen and other nutrients. On the other hand, heterotrophic organisms such as bacteria and fungi do not require large amounts of (sun)light and can recover waste COD as well. Coppens (2016) compared the CAPEX and OPEX costs of SCP production installations using different types of organisms, and found that, per unit of nitrogen recovered, the combined costs for SCP production by heterotrophic organisms was 4 to 30 times less expensive compared to autotrophic SCP production by hydrogen oxidizing bacteria, purple non-sulfur bacteria, and microalgae. Recent studies considered the production of SCP from food processing wastewaters (Lee et al., 2015; Liu et al., 2016). However, considering SCP from municipal wastewater, the safest way to avoid potential contamination with pathogens and pollutants is to recover energy and nutrients indirectly, for example by generating hydrogen and CO_2 by reformation of biogas produced from sludge digestion, recovering ammonia-nitrogen by air stripping of the digestate, and subsequently producing SCP from the relatively pure streams of hydrogen and ammonia by means of hydrogen oxidizing bacteria (Matassa et al., 2015). In such a wastewater treatment scheme, the HiCS process may prove its value over conventional technologies because of its high redirection of COD and nitrogen to sludge.

Any technology that recovers resources from anaerobic digestate, may be used complimentary to the HRAS and AD processes for energy recovery. For example, ammonia can be recovered from digestate by air stripping (Desloover et al., 2012a; Siegrist, 1996), and both ammonia and phosphorus can be recovered by struvite precipitation (Bhuiyan et al., 2008; Siegrist, 1996). After dewatering, the solid fraction of the digestate can be dried and pyrolyzed at low temperature to immobilize the residual carbon and organic phosphorus in the form of biochar (Verstraete & Vlaeminck, 2011). Biochar is a

charcoal-like material that can be used to improve the structure and fertility of soils because it can retain and slowly release fertilizer minerals (Lehmann, 2007). Biochar from municipal WWTP sludge, however, may pose a number of health risks. Domestic wastewater typically contains a range of heavy metals, such as copper, zinc, lead, and mercury (Sörme & Lagerkvist, 2002). Because biochar from municipal WWTPs may contain part of these heavy metals, careful monitoring, control and remediation may be necessary before it can be applied to land (Verstraete & Vlaeminck, 2011).

Technologies that require COD as a substrate may potentially interfere with optimal energy recovery from wastewater. As discussed in Chapter 5, a relatively large amount of COD is needed to achieve nitrogen removal by the conventional process of nitrification/denitrification (N/DN), but the COD requirement can be lowered by adopting novel technologies such as nitritation/denitritation (nit/denit) or partial nitritation/anammox (PN/A). As discussed in Chapter 1, enhanced biological phosphorus removal (EBPR) requires cyclic exposure of the sludge to anaerobic and anoxic / aerobic conditions. Because the phosphate accumulating organisms (PAOs) require rapidly biodegradable COD such as volatile fatty acids (VFA) during the anaerobic phase, EBPR is typically performed at the beginning of the biological treatment process, when rapidly biodegradable COD is still present. As such, it may be difficult to integrate EBPR with maximal recovery of COD in an A-B-type plant configuration. On the other hand, phosphorus removal by means of chemical precipitation does not have a COD requirement.

From a COD-centered perspective, the choice between carbon, nitrogen and phosphorus removal technologies impacts the overall balance, and thus determines the maximal amount of COD that can be recovered as energy or bioproducts. In this point of view, COD in wastewater should be considered a limited resource, and the choice for one technology that consumes COD will limit its availability for other processes. In the traditional approach of wastewater treatment, COD is removed by destruction, i.e., most of the COD that is not removed in the processes of sludge production, denitrification, and EBPR, is removed by aerobic oxidation. With the aim to maximize COD recovery, alternative technologies should be selected that remove carbon, nitrogen and phosphorus with minimal destructive losses of COD. Differences in COD balance between the 'traditional' and 'new' approach to wastewater treatment are schematized in **Figure 6.6**.

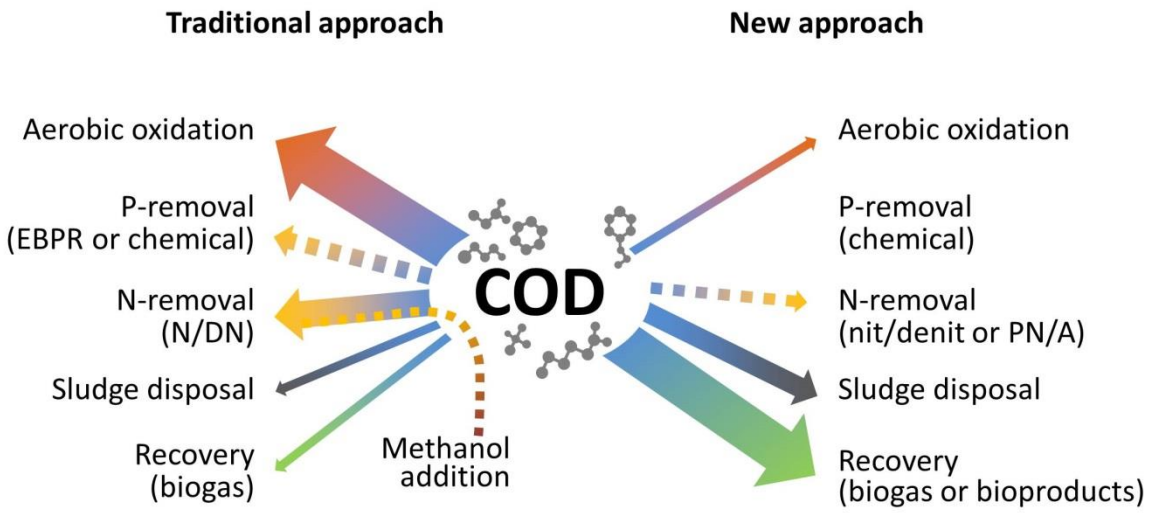


Figure 6.6: Wastewater COD as a limited resource. The fate of COD in different wastewater treatment processes, according to the 'traditional' (left) and 'new' approach (right). The thickness of the arrows represents a qualitative indication of mass flux. Dashed arrows indicate that COD consumption is possible, depending on the specific technology.

At all times, economic factors will play a decisive role in the choice between technologies. Combining technologies for maximum COD recovery does not necessarily lead to the most economic overall process. Starting from the 'traditional' situation depicted in **Figure 6.6**, initial improvements in COD recovery may lead to lower operational costs due to a higher energy production from biogas. However, at a certain point, a trade-off may emerge in which additional improvements in COD recovery lead to increased overall costs. For example, addition of chemicals may increase recovery of COD by improving bioflocculation or eliminating the need for EBPR, but chemicals may significantly contribute to a plant's overall costs (see Chapter 5). Cost optimization and technological approaches for nitrogen and phosphorus recovery should be considered together with the optimization of COD recovery in order to design a more sustainable wastewater treatment scheme for the future.

6 Conclusion

This work aimed to address the inefficiencies of conventional wastewater treatment in terms of energy consumption and removal of COD with limited recovery. High-rate activated sludge technologies were explored, in order to increase sludge production during primary wastewater treatment, and improve the potential for recovery of energy or bioproducts from wastewater. The microbial ecology of a full-scale high-rate system was compared to that of a conventional, low-rate system (Chapter 2), where it was found that high-rate communities are more variable over time, and that in high-rate communities, neutral changes play a larger role in this variability than changes as a

result of environmental factors. This may result in a lower predictability or 'controllability' of high-rate communities compared to low-rate communities, and may have implications for the development of process control strategies. More research is needed to connect microbial community structure to functional output of high-rate systems.

In an effort to improve the biosorption capacity (i.e., accumulation, adsorption and storage) of high-rate activated sludge, a high-rate contact stabilization (HiCS) configuration was proposed as a modification of the conventional high-rate systems (HiCAS) currently in use. Chapters 3 and 4 compared the performance of the HiCS process to that of the HiCAS process in laboratory-scale reactors and optimized the SRT, stabilization time and contact time of the HiCS process for maximal COD recovery. Over the course of the different experiments performed, the HiCS process was always able to achieve a similar amount of COD recovery compared to the HiCAS process, but with lower losses of COD by aerobic oxidation. Storage played a minor role in the overall removal of COD, which could mainly be attributed to bioflocculation and cellular growth. Subsequently, Chapter 5 explored the implications of different technologies for primary and secondary treatment, including the HiCS process, on the performance of a full-scale wastewater treatment plant, in terms of the overall energy consumption, and operational and investment costs. The HiCS process, combined with any of the nitrogen removal technologies in the secondary stage, achieved the lowest overall operational costs at near-energy neutrality, and the capital costs for constructing a HiCS installation could be compensated for by the savings in operational costs compared to a stand-alone CAS system. Not considering the need for chemical addition, the main advantage of the HiCS system over conventional HiCAS was the lower volume requirement of the reactors.

Overall, the HiCS process shows a promising potential to improve the overall energy balance of wastewater treatment. The main advantages of the HiCS process seem to be that it achieves an acceptable level of COD redirection to sludge with limited oxygen requirements and reactor volumes that are substantially lower than those of conventional high-rate systems. The effluent quality of the HiCS system is, however, a point where further optimization is needed. A relatively large fraction of particulate and dissolved COD are washed out of the system, which results in higher downstream aeration requirements, and demonstrates that the redirection of COD to sludge is not complete.

Further research should focus on improving bioflocculation capacity and solid/liquid separation in the HiCS process, especially for medium to low-strength wastewater, and assess its compatibility with other technologies for resource recovery from wastewater. As of currently, no single set of optimal operational conditions can be recommended for the HiCS system, as performance is dependent on influent conditions (see Chapter 5). In order to move the technology forward, a more profound understanding is needed of the mechanisms of adsorption, storage, growth and oxidation in the HiCS system, as well as the influence of operational variables on these processes. Further research should be performed with the aim of developing a decision-making framework for HiCS operation, depending on conditions such as influent strength, composition, and quality requirements of the effluent for downstream treatment. In the quest for a more sustainable water and wastewater

cycle, economic factors remain the foremost drivers of change. For each combination of technologies, a balance should be made between the benefits of recovering COD, nutrients and other resources on the one hand, and the potential disadvantages of increased costs, contamination of reused resources, and environmental impacts on the other hand.

REFERENCES

ABEYSINGHE, D. H., DE SILVA, D. G. V., STAHL, D. A. & RITTMANN, B. E. (2002). The effectiveness of bioaugmentation in nitrifying systems stressed by a washout condition and cold temperature. *Water Environment Research* **74**(2): 187-199.

ADAMSKI, R. E., DESANTIS, V., SPANGEL, A., PYNN, J., BETTY, L., KOCH, C. M. & GYORY, S. A. (2000). Step-feed operation at short detention times – A cost effective method for improving wastewater treatment. *Water Science and Technology* **41**(9): 15-20.

AGLER, M. T., WRENN, B. A., ZINDER, S. H. & ANGENENT, L. T. (2011). Waste to bioproduct conversion with undefined mixed cultures: The carboxylate platform. *Trends in Biotechnology* **29**(2): 70-78.

AIYUK, S. & VERSTRAETE, W. (2004). Sedimentological evolution in an UASB treating SYNTHES, a new representative synthetic sewage, at low loading rates. *Bioresource Technology* **93**(3): 269-278.

AKANYETI, I., TEMMINK, H., REMY, M. & ZWIJNENBURG, A. (2010). Feasibility of bioflocculation in a high-loaded membrane bioreactor for improved energy recovery from sewage. *Water Science and Technology* **61**(6): 1433–1439.

ALBERTSEN, M., STENSBALLE, A., NIELSEN, K. L. & NIELSEN, P. H. (2013). Digging into the extracellular matrix of a complex microbial community using a combined metagenomic and metaproteomic approach. *Water Science and Technology* **67**(7): 1650-1656.

ALCAMO, J., FLÖRKE, M. & MÄRKER, M. (2007). Future long-term changes in global water resources driven by socio-economic and climatic changes. *Hydrological Sciences Journal* **52**(2): 247-275.

ANDERSEN, S. J., CANDRY, P., BASADRE, T., KHOR, W. C., ROUME, H., HERNANDEZ-SANABRIA, E., COMA, M. & RABAEY, K. (2015). Electrolytic extraction drives volatile fatty acid chain elongation through lactic acid and replaces chemical pH control in thin stillage fermentation. *Biotechnology for Biofuels* **8**(1): 1-14.

ANGENENT, L. T., KARIM, K., AL-DAHHAN, M. H., WRENN, B. A. & DOMÍGUEZ-ESPINOSA, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology* **22**(9): 477-485.

ANUPAMA & RAVINDRA, P. (2000). Value-added food: Single cell protein. *Biotechnology Advances* **18**(6): 459-479.

AQUASTAT. (2014). The Food and Agriculture Organization of the United Nations (FAO) global water information system. (Available at http://www.fao.org/nr/water/aquastat/main/index.stm). (Accessed 25 Apr. 2016).

ARDERN, E. & LOCKETT, W. T. (1914). Experiments on the oxidation of sewage without the aid of filters. *Journal of the Society of the Chemical Industry* **33**: 523-539.

AßHAUER, K. P., WEMHEUER, B., DANIEL, R. & MEINICKE, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* **31**(17): 2882-2884.

AYARZA, J. M. & ERIJMAN, L. (2011). Balance of neutral and deterministic components in the dynamics of activated sludge floc assembly. *Microbial Ecology* **61**(3): 486-495.

BAGCHI, S., TELLEZ, B. G., RAO, H. A., LAMENDELLA, R. & SAIKALY, P. E. (2015). Diversity and dynamics of dominant and rare bacterial taxa in replicate sequencing batch reactors operated under different solids retention time. *Applied Microbiology and Biotechnology* **99**(5): 2361-2370.

BANDYOPADHYAY, B., HUMPHREY, A. E., TAGUCHI, H. & INTRODUCTION BY G. RAO. (2009). Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems. *Biotechnology and Bioengineering* **104**(5): 841-853.

BARBERAN, A., BATES, S. T., CASAMAYOR, E. O. & FIERER, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *The ISME journal* **6**(2): 343-351.

BARNARD, J. L. (1973). Biological denitrification. *Water Pollution Control* **72**(6): 705-720.

BARNES, D. & BLISS, P. J. (1983). *Biological control of nitrogen in wastewater treatment*. E. & F.N. Spon, London, U.K.

BARR, T. A., TAYLOR, J. M. & DUFF, S. J. B. (1996). Effect of HRT, SRT and temperature on the performance of activated sludge reactors treating bleached kraft mill effluent. *Water Research* **30**(4): 799-810.

BECCARI, M., MAJONE, M., MASSANISSO, P. & RAMADORI, R. (1998). A bulking sludge with high storage response selected under intermittent feeding. *Water Research* **32**(11): 3403-3413.

BENEFIELD, L. D. & RANDALL, C. W. (1976). Design procedure for a contact stabilization activated sludge process. *Journal - Water Pollution Control Federation* **48**(1): 147-152.

BERRY, D. & WIDDER, S. (2014). Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology* **5**: 219.

BEUN, J. J., DIRCKS, K., VAN LOOSDRECHT, M. C. M. & HEIJNEN, J. J. (2002). Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. *Water Research* **36**(5): 1167-1180.

BEUN, J. J., HENDRIKS, A., VAN LOOSDRECHT, M. C. M., MORGENROTH, E., WILDERER, P. A. & HEIJNEN, J. J. (1999). Aerobic granulation in a sequencing batch reactor. *Water Research* **33**(10): 2283-2290.

BHUIYAN, M. I., MAVINIC, D. S. & KOCH, F. A. (2008). Phosphorus recovery from wastewater through struvite formation in fluidized bed reactors: a sustainable approach. *Water Science and Technology* **57**(2): 175-181.

BLACKBURNE, R., VADIVELU, V. M., YUAN, Z. & KELLER, J. (2007a). Determination of growth rate and yield of nitrifying bacteria by measuring carbon dioxide uptake rate. *Water Environment Research* **79**(12): 2437-2445.

BLACKBURNE, R., VADIVELU, V. M., YUAN, Z. & KELLER, J. (2007b). Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. *Water Research* **41**(14): 3033-3042.

BÖHNKE, B. (1977). Das Adsorptions-Belebungsverfahren (*in German*). *Korrespondenz Abwasser* **24**(2): 33-42.

BÖHNKE, B. (1984). Reaktionsabläufe in der A-Stufe als Funktion von Variabilität, Selektion und Mutation sowie von der Konjugation (*in German*). In *A-B-Technologie : Erfahrungsaustausch*. Gesellschaft zur Förderung der Siedlungswasserwirtschaft, 26 - 28 September 1983, Aachen, Germany.

BÖHNKE, B. (1985). Mikrobiologische Reaktionsabläufe beim AB-Verfahren (*in German*). *Acta Biotechnologica* **5**(1): 45-50.

BÖHNKE, B., DIERING, B. & ZUCKUT, S. W. (1997a). AB Process removes organics and nutrients. *Water Environment & Technology* **9**(3): 23-27.

BÖHNKE, B., DIERING, B. & ZUCKUT, S. W. (1997b). Cost-effective wastewater treatment process for removal of organics and nutrients (part I). *Water Engineering & Management* **144**(5): 30-34.

BÖHNKE, B., DIERING, B. & ZUCKUT, S. W. (1997c). Cost-effective wastewater treatment process for removal of organics and nutrients (part II). *Water Engineering & Management* **144**(7): 18-21.

BOHORQUEZ, L. C., DELGADO-SERRANO, L., LÓPEZ, G., OSORIO-FORERO, C., KLEPAC-CERAJ, V., KOLTER, R., JUNCA, H., BAENA, S. & ZAMBRANO, M. M. (2012). In-depth characterization via complementing culture-independent approaches of the microbial community in an acidic hot spring of the Colombian Andes. *Microbial Ecology* **63**(1): 103-115.

BOLZONELLA, D., PAVAN, P., BATTISTONI, P. & CECCHI, F. (2005). Mesophilic anaerobic digestion of waste activated sludge: Influence of the solid retention time in the wastewater treatment process. *Process Biochemistry* **40**(3–4): 1453-1460.

BRATBY, J. (2006). *Coagulation and flocculation in water and wastewater treatment – Third edition*. IWA Publishing, London, U.K. 538 pp.

BRIONES, A. & RASKIN, L. (2003). Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. *Current Opinion in Biotechnology* **14**(3): 270-276.

BUNCH, B. & GRIFFIN, D. M. J. (1987). Rapid removal of colloidal substrate from domestic wastewaters. *Journal - Water Pollution Control Federation* **59**(11): 957-963.

BUSWELL, A. M. & LONG, H. L. (1923). Microbiology and theory of activated sludge. *Journal - American Water Works Association* **10**(2): 309-321.

CABIROL, N., BARRAGAN, E. J., DURAN, A. & NOYOLA, A. (2003). Effect of aluminium and sulphate on anaerobic digestion of sludge from wastewater enhanced primary treatment. *Water Science and Technology* **48**(6): 235-40.

CACCAVO, F., FROLUND, B., VAN OMMEN, K. F. & NIELSEN, P. H. (1996). Deflocculation of activated sludge by the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY. *Applied and Environmental Microbiology* **62**(4): 1487-1490.

CAGNETTA, C., COMA, M., VLAEMINCK, S. E. & RABAEY, K. (2016). Production of carboxylates from high rate activated sludge through fermentation. *Bioresource Technology*: dx.doi.org/10.1016/j.biortech.2016.03.053.

CAMARINHA-SILVA, A., JÁUREGUI, R., CHAVES-MORENO, D., OXLEY, A. P. A., SCHAUMBURG, F., BECKER, K., WOS-OXLEY, M. L. & PIEPER, D. H. (2014). Comparing the anterior nare bacterial community of two discrete human populations using Illumina amplicon sequencing. *Environmental Microbiology* **16**(9): 2939-2952.

CAMPBELL, B. J., YU, L., HEIDELBERG, J. F. & KIRCHMAN, D. L. (2011). Activity of abundant and rare bacteria in a coastal ocean. *Proceedings of the National Academy of Sciences of the United States of America* **108**(31): 12776-12781.

CBO. (2002). Future investment in drinking water and wastewater infrastructure. A Congressional Budget Office study (Available at https://www.cbo.gov/publication/14205).

CECH, J. S. & CHUDOBA, J. (1983). Influence of accumulation capacity of activated of activated sludge microorganisms on kinetics of glucose removal. *Water Research* **17**(6): 659-666.

CECH, J. S., CHUDOBA, J. & GRAU, P. (1985). Determination of kinetic constants of activated-sludge microorganisms. *Water Science and Technology* **17**(2-3): 259-272.

CHAO, A. C. & KEINATH, T. M. (1979). Influence of process loading intensity on sludge clarification and thickening characteristics. *Water Research* **13**(12): 1213-1223.

CHASE, J. M. & LEIBOLD, M. A. (2003). *Ecological niches: Linking classical and contemporary approaches*. University of Chicago Press, Chicago, IL, U.S.A.

CHUDOBA, J., DOHÁNYOS, M. & GRAU, P. (1982). Control of activated sludge filamentous bulking — IV. Effect of sludge regeneration. *Water Science and Technology* **14**(1-2): 73-93.

CHUDOBA, J., GRAU, P. & OTTOVA, V. (1973). Control of activated-sludge filamentous bulking - II. Selection of microorganisms by means of a selector. *Water Research* **7**(10): 1389-1398.

CLARK, H. W. & ADAMS, G. O. (1914). Sewage treatment by aeration and contact in tanks containing layers of slate. *Engineering Record* (69): 158-159.

CLARK, H. W. & GAGE, S. DE M. (1913). Work of the Lawrence Experiment Station for 1913 – the purification of sewage and investigation on allied subjects. 45th annual report, pp. 265–368. State Board of Health, Massachusetts, U.S.A.

COLE, J. R., WANG, Q., FISH, J. A., CHAI, B., MCGARRELL, D. M., SUN, Y., BROWN, C. T., PORRAS-ALFARO, A., KUSKE, C. R. & TIEDJE, J. M. (2014). Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research* **42**(Database issue): D633-42.

COLLIVER, B. B. & STEPHENSON, T. (2000). Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnology Advances* **18**(3): 219-232.

CONSTANTINE, T., HOUWELING, D. & KRAEMER, J. T. (2012). "Doing the two-step" - Reduced energy consumption sparks renewed interest in multistage biological treatment. In *Proceedings of the 85th Water Environment Federation Technical Exhibition and Conference (WEFTEC)*, 29 September - 3 October 2012, New Orleans, LA, U.S.A.

COOMBS, J. A. (1921). Improvements in or connected with the treatment of sewage and other impure liquids, pp. 14, United Kingdom Patent 187315-A.

COPPENS, J. (2016). Quantification, recovery and valorization of nitrogen and phosphorus from waste streams: strategies towards circular nutrient management. PhD thesis, Laboratory of Microbial Ecology and Technology (LabMET), Ghent University.

CORNEL, P., MEDA, A. & BIEKER, S. (2011). Wastewater as a source of energy, nutrients and service water. In *Treatise on Water Science* (ed. P. Wilderer), pp. 337-375. Elsevier Verlag, Oxford.

COURTENS, E. (2015). Exploring the boundaries of biological nitrogen removal: Development strategies for thermophilic nitrification and denitrification. PhD thesis, Laboratory of Microbial Ecology and Technology (LabMET), Ghent University.

COURTENS, E. N. P., VLAEMINCK, S. E., VILCHEZ-VARGAS, R., VERLIEFDE, A., JAUREGUI, R., PIEPER, D. H. & BOON, N. (2014). Trade-off between mesophilic and thermophilic denitrification: Rates vs. sludge production, settleability and stability. *Water Research* **63**: 234-244.

CROCETTI, G. R., HUGENHOLTZ, P., BOND, P. L., SCHULER, A., KELLER, J., JENKINS, D. & BLACKALL, L. L. (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Applied and Environmental Microbiology* **66**(3): 1175-1182.

CUEVAS, D. A., EDIRISINGHE, J., HENRY, C. S., OVERBEEK, R., O'CONNELL, T. G. & EDWARDS, R. A. (2016). From DNA to FBA: How to build your own genome-scale metabolic model. *Frontiers in Microbiology* **7**(907).

CURTIS, T. P., HEAD, I. M. & GRAHAM, D. W. (2003). Peer reviewed: Theoretical ecology for engineering biology. *Environmental Science & Technology* **37**(3): 64A-70A.

DAIGGER, G. T. & GRADY, C. P. L., JR. (1982). The dynamics of microbial growth on soluble substrates: A unifying theory. *Water Research* **16**(4): 365-382.

DAIMS, H., LEBEDEVA, E. V., PJEVAC, P., HAN, P., HERBOLD, C., ALBERTSEN, M., JEHMLICH, N., PALATINSZKY, M., VIERHEILIG, J., BULAEV, A., KIRKEGAARD, R. H., VON BERGEN, M., RATTEI, T., BENDINGER, B., NIELSEN, P. H. & WAGNER, M. (2015). Complete nitrification by Nitrospira bacteria. *Nature* **528**(7583): 504-509.

DC WATER. (2016). Tours of Blue Plains (Available at https://www.dcwater.com/about/tours.cfm). (Accessed 4 May 2016).

DE CLIPPELEIR, H., VLAEMINCK, S., COURTENS, E., VERSTRAETE, W. & BOON, N. (2013a). Oxygen-limited autotrophic nitrification/denitrification maximizes net energy gain in technology schemes with anaerobic digestion. In *Renewable Energy Sources*. Academy Publish, Wyoming, USA.

DE CLIPPELEIR, H., VLAEMINCK, S. E., COURTENS, E. N. P., JIMENEZ, J., WADHAWAN, T. & ZHANG, Q. (2015). Towards energy autarky : Carbon redirection coupled with shortcut nitrogen processes. In *Shortcut nitrogen removal – Nitrite shunt and deammonification*. Water Environment Federation (WEF), Alexandria, U.S.A.

DE CLIPPELEIR, H., VLAEMINCK, S. E., DE WILDE, F., DAENINCK, K., MOSQUERA, M., BOECKX, P., VERSTRAETE, W. & BOON, N. (2013b). One-stage partial nitritation/anammox at 15 °C on pretreated sewage: Feasibility demonstration at lab-scale. *Applied Microbiology and Biotechnology* **97**(23): 10199-10210.

DE GRAAFF, M. & ROEST, K. (2012). Inventarisatie van AB-systemen - Optimale procescondities in de A-trap (*in Dutch*). *KWR rapport 2012-094* pp. 65.

DE LOS REYES III, F. L. (2010). Challenges in determining causation in structure–function studies using molecular biological techniques. *Water Research* **44**(17): 4948-4957.

DE SMEDT, V. (2015). The high-rate contact stabilisation system: Combining experiments and modelling to gain system knowledge. Master thesis, LabMET - BIOMATH, Universiteit Gent, Belgium.

DE VRIEZE, J., DE LATHOUWER, L., VERSTRAETE, W. & BOON, N. (2013). High-rate iron-rich activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste. *Water Research* **47**(11): 3732-3741.

DEARMOND, J., MILLER, M. W., KINNEAR, D., WETT, B., MURTHY, S. & BOTT, C. B. (2015). Quantifying sludge handling characteristics of an adsorption style HRAS process which precedes an advanced shortcut nitrogen process. In *WEF/IWA Residuals and Biosolids conference*, 7 - 10 June 2015, Washington, D.C. (U.S.A.).

DECUBBER, S. (2014). Linking the carbon biokinetics of activated sludge to the operational wastewater treatment conditions Master thesis, LabMET, Ghent University.

DESLOOVER, J., ABATE WOLDEYOHANNIS, A., VERSTRAETE, W., BOON, N. & RABAEY, K. (2012a). Electrochemical resource recovery from digestate to prevent ammonia toxicity during anaerobic digestion. *Environmental Science & Technology* **46**(21): 12209-12216.

DESLOOVER, J., VLAEMINCK, S. E., CLAUWAERT, P., VERSTRAETE, W. & BOON, N. (2012b). Strategies to mitigate N2O emissions from biological nitrogen removal systems. *Current Opinion in Biotechnology* **23**(3): 474-482.

DIAMANTIS, V., VERSTRAETE, W., EFTAXIAS, A., BUNDERVOET, B., SIEGFRIED, V., MELIDIS, P. & AIVASIDIS, A. (2013). Sewage pre-concentration for maximum recovery and reuse at decentralized level. *Water Science and Technology* **67**(6): 1188-1193.

DING, H.-B., DOYLE, M., ERDOGAN, A., WIKRAMANAYAKE, R. & GALLAGHER, P. (2015a). Innovative use of dissolved air flotation with biosorption as primary treatment to approach energy neutrality in WWTPs. *Water Practice & Technology* **10**(1): 133-142.

DING, Z., BOURVEN, I., GUIBAUD, G., VAN HULLEBUSCH, E. D., PANICO, A., PIROZZI, F. & ESPOSITO, G. (2015b). Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. *Applied Microbiology and Biotechnology* **99**(23): 9883-9905.

DIONISI, H. M., LAYTON, A. C., HARMS, G., GREGORY, I. R., ROBINSON, K. G. & SAYLER, G. S. (2002). Quantification of Nitrosomonas oligotropha-Like Ammonia-Oxidizing Bacteria and Nitrospira spp. from Full-Scale Wastewater Treatment Plants by Competitive PCR. *Applied and Environmental Microbiology* **68**(1): 245-253.

DIRCKS, K., PIND, P. F., MOSBÆK, H. & HENZE, M. (1999). Yield determination by respirometry - The possible influence of storage under aerobic conditions in activated sludge. *Water SA* **25**(1): 69-74.

DIRECTIVE 91/271/EEC. Council Directive (15 May 1991) concerning urban waste water treatment, OJ L 135 of 30 May 1991, amended by Commission Directive 98/15/EC of 27 February 1998, OJ L 67 of 7 March 1998.

DOYLE, J. D. & PARSONS, S. A. (2002). Struvite formation, control and recovery. *Water Research* **36**(16): 3925-3940.

DUAN, L., MORENO-ANDRADE, I., HUANG, C.-L., XIA, S. & HERMANOWICZ, S. W. (2009). Effects of short solids retention time on microbial community in a membrane bioreactor. *Bioresource Technology* **100**(14): 3489-3496.

DUGAN, P. R., STONER, D. L. & PICKRUM, H. M. (2006). The genus *Zoogloea*. In *The Prokaryotes* (ed. M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt). Springer-Verlag, New York, U.S.A.

DWORKIN, M., FALKOW, S., ROSENBERG, E., SCHLEIFER, K.-H. & STACKEBRANDT, E. (EDS). (2006). *The Prokaryotes: A Handbook on the Biology of Bacteria.*, Third edition. Springer-Verlag, New York.

EKAMA, G., BARNARD, J., GUNTHERT, F., KREBS, P., MCCORQUODALE, J. & PARKER, D. (1997). *Secondary settling tanks: Theory, modelling, design and operation*. International Association on Water Quality, London, UK.

ETCHEBEHERE, C., CABEZAS, A., DABERT, P. & MUXI, L. (2003). Evolution of the bacterial community during granules formation in denitrifying reactors followed by molecular, culture-independent techniques. *Water Science and Technology* **48**(6): 75-9.

EUROSTAT. (2015). Energy price statistics (Available at http://ec.europa.eu/eurostat/statistics-explained/index.php/Energy_price_statistics). (Accessed 29 Apr. 2016).

FAUST, L., SZENDY, M., PLUGGE, C. M., VAN DEN BRINK, P. F., TEMMINK, H. & RIJNAARTS, H. H. (2015). Characterization of the bacterial community involved in the bioflocculation process of wastewater organic matter in high-loaded MBRs. *Applied Microbiology and Biotechnology* **99**(12): 5327-5337.

FAUST, L., TEMMINK, H., ZWIJNENBURG, A., KEMPERMAN, A. J. B. & RIJNAARTS, H. H. M. (2014a). Effect of dissolved oxygen concentration on the bioflocculation process in high loaded MBRs. *Water Research* **66**(0): 199-207.

FAUST, L., TEMMINK, H., ZWIJNENBURG, A., KEMPERMAN, A. J. B. & RIJNAARTS, H. H. M. (2014b). High loaded MBRs for organic matter recovery from sewage: Effect of solids retention time on bioflocculation and on the role of extracellular polymers. *Water Research* **56**: 258-266.

FONTS, I., GEA, G., AZUARA, M., ÁBREGO, J. & ARAUZO, J. (2012). Sewage sludge pyrolysis for liquid production: A review. *Renewable and Sustainable Energy Reviews* **16**(5): 2781-2805.

FRØLUND, B., GRIEBE, T. & NIELSEN, P. H. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology* **43**(4): 755-761.

GAO, H., SCHERSON, Y. D. & WELLS, G. F. (2014). Towards energy neutral wastewater treatment: Methodology and state of the art. *Environmental Science: Processes & Impacts* **16**(6): 1223-1246.

GE, H., BATSTONE, D. J. & KELLER, J. (2013). Operating aerobic wastewater treatment at very short sludge ages enables treatment and energy recovery through anaerobic sludge digestion. *Water Research* **47**(17): 6546-6557.

GE, H., BATSTONE, D. J. & KELLER, J. (2015). Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO clade Comamonadaceae. *Water Research* **69**(0): 173-182.

GENTILE, M. E., JESSUP, C. M., NYMAN, J. L. & CRIDDLE, C. S. (2007). Correlation of Functional Instability and Community Dynamics in Denitrifying Dispersed-Growth Reactors. *Applied and Environmental Microbiology* **73**(3): 680-690.

GERARDI, M. H. (2003). *Settleability problems and loss of solids in the activated sludge process*. John Wiley & Sons, New Jersey, U.S.A.

GERHARDT, P., MURRAY, R. G. E., WOOD, W. A. & KRIEG, N. R., EDS. (1994). *Methods for General and Molecular Bacteriology*. American Society for Microbiology, Washington, D.C., U.S.A. 791 pp.

GERNAEY, K., PETERSEN, B., NOPENS, I., COMEAU, Y. & VANROLLEGHEM, P. A. (2002). Modeling aerobic carbon source degradation processes using titrimetric data and combined respirometric–titrimetric data: Experimental data and model structure. *Biotechnology and Bioengineering* **79**(7): 741-753.

GETHKE, H.-G. (1984). Übersicht über Versuchsergebnisse zum A-B-Verfahren (*in German*). In *A-B-Technologie : Erfahrungsaustausch*. Gesellschaft zur Förderung der Siedlungswasserwirtschaft, 26 - 28 September 1983, Aachen, Germany.

GOBET, A., QUINCE, C. & RAMETTE, A. (2010). Multivariate cutoff level analysis (MultiCoLA) of large community data sets. *Nucleic Acids Research* **38**(15): e155.

GONZALEZ-MARTINEZ, A., RODRIGUEZ-SANCHEZ, A., LOTTI, T., GARCIA-RUIZ, M.-J., OSORIO, F., GONZALEZ-LOPEZ, J. & VAN LOOSDRECHT, M. C. M. (2016). Comparison of bacterial communities of conventional and A-stage activated sludge systems. *Scientific Reports* **6**: 18786.

GONZALEZ, R., CAMPBELL, P. & WONG, M. (2010). Production of ethanol from thin stillage by metabolically engineered *Escherichia coli*. *Biotechnology Letters* **32**(3): 405-411.

GOSSETT, J. M., MCCARTY, P. L., WILSON, J. C. & EVANS, D. S. (1978). Anaerobic digestion of sludge from chemical treatment. *Journal - Water Pollution Control Federation* **50**(3): 533-542.

GRADY, C. P. L., SMETS, B. F. & BARBEAU, D. S. (1996). Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. *Water Research* **30**(3): 742-748.

GRAY, D. M. D., LANGE, V. P. D., CHIEN, M. H., ESQUER, M. A. & SHAO, Y. J. (2006). Investigating the fundamental basis for selectors to improve activated sludge settling. In *Proceedings of the 79th Water Environment Federation Technical Exhibition and Conference (WEFTEC)*, 22-25 October, Dallas, TA, U.S.A.

GREENBERG, A. E., CLESCERI, L. S. & EATON, A. D. (EDS). (1992). *Standard methods for the examination of water and wastewater*. American Public Health Association Publications, Washington, D.C., U.S.A.

GRIFFITH, J. W. & RAYMOND, D. H. (2002). The first commercial supercritical water oxidation sludge processing plant. *Waste Management* **22**(4): 453-459.

GRISSOP, G. (2010). Biological nutrient removal processes. Shenandoah Valley Pure Water Forum.

GUELLIL, A., THOMAS, F., BLOCK, J. C., BERSILLON, J. L. & GINESTET, P. (2001). Transfer of organic matter between wastewater and activated sludge flocs. *Water Research* **35**(1): 143-150.

GUEST, J. S., SKERLOS, S. J., BARNARD, J. L., BECK, M. B., DAIGGER, G. T., HILGER, H., JACKSON, S. J., KARVAZY, K., KELLY, L., MACPHERSON, L., MIHELCIC, J. R., PRAMANIK, A., RASKIN, L., VAN LOOSDRECHT, M. C. M., YEH,

D. & LOVE, N. G. (2009). A new planning and design paradigm to achieve sustainable resource recovery from wastewater. *Environmental Science & Technology* **43**(16): 6126-6130.

GUJER, W. & JENKINS, D. (1975). The contact stabilization activated sludge process - oxygen utilization, sludge production and efficiency. *Water Research* **9**(5-6): 553-560.

HAI, R., WANG, Y., WANG, X., LI, Y. & DU, Z. (2014). Bacterial community dynamics and taxa-time relationships within two activated sludge bioreactors. *PLoS ONE* **9**(3): 8 pp.

HAIDER, S. (2002). CSB-Elimination in höchstbelasteten Belebungsstufen und ihre Auswirkung auf die Stickstoffelimination von zweistufigen Anlagen unter dem Gesichtspunkt der mathematischen Modellierung (*in German*). *Wiener Mitteilungen Band 188*. PhD thesis, Institut f. Wassergüte, Ressourcenmanagement u. Abfallwirtschaft, TU Wien, Austria.

HAIDER, S., SVARDAL, K., VANROLLEGHEM, P. A. & KROISS, H. (2003). The effect of low sludge age on wastewater fractionation (S(S), S(I)). *Water Science and Technology* **47**(11): 203-209.

HAIDER, S., VANROLLEGHEM, P. A. & KROIß, H. (2000). Low sludge age and its consequences for metabolisation, storage and adsorption of readily biodegradable substrate (Ss). In *Proceedings 1st IWA World Water Congress*, 3 - 7 July 2000, Paris, France.

HANEMAAIJER, M., RÖLING, W. F. M., OLIVIER, B. G., KHANDELWAL, R. A., TEUSINK, B. & BRUGGEMAN, F. J. (2015). Systems modeling approaches for microbial community studies: from metagenomics to inference of the community structure. *Frontiers in Microbiology* **6**: 213.

HARRELL, F. E., JR. (2014). Hmisc: Harrell Miscellaneous. *R package version 3.14-6.* (Available at http://CRAN.R-project.org/package=Hmisc).

HATfiELD, W. D. (1959). Modified plant processes to handle shock loadings. *Wastes Engineering* **30**: 612-619.

HAUGAARD MIKKELSEN, L. & KEIDING, K. (1999). Equilibrium aspects of the effects of shear and solids content on aggregate deflocculation. *Advances in Colloid and Interface Science* **80**(2): 151-182.

HAYDAR, S. & AZIZ, J. A. (2009). Characterization and treatability studies of tannery wastewater using chemically enhanced primary treatment (CEPT)—A case study of Saddiq Leather Works. *Journal of Hazardous Materials* **163**(2–3): 1076-1083.

HEIDRICH, E. S., CURTIS, T. P. & DOLFING, J. (2011). Determination of the internal chemical energy of wastewater. *Environmental Science & Technology* **45**(2): 827-832.

HENZE, M., GUJER, W., MINO, T. & LOOSDRECHT, M. V. (2000). Activated sludge models ASM1, ASM2, ASM2d and ASM3. Scientific and Technical report No.9. IWA Task Group on Mathematical Modeling for Design and Operation of Biological Wastewater Treatment.

HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (EDS). (2008). *Biological Wastewater Treatment: Principles, Modelling and Design*. IWA publishing, London, U.K.

HERNANDEZ LEAL, L., TEMMINK, H., ZEEMAN, G. & BUISMAN, C. J. (2010). Bioflocculation of grey water for improved energy recovery within decentralized sanitation concepts. *Bioresource Technology* **101**(23): 9065-9070.

HESHAM, A. E.-L., QI, R. & YANG, M. (2011). Comparison of bacterial community structures in two systems of a sewage treatment plant using PCR-DGGE analysis. *Journal of Environmental Sciences (China)* **23**(12): 2049-2054.

HOOVER, S. R. & PORGES, N. (1952). Assimilation of dairy wastes by activated sludge: II. The equation of synthesis and rate of oxygen utilization. *Sewage and Industrial Wastes* **24**(3): 306-312.

HOU, P.-B., LI, Y.-Z., WU, B.-H., YAN, Z.-C., YAN, B.-X. & GAO, P.-J. (2006). Cellulolytic complex exists in cellulolytic myxobacterium *Sorangium*. *Enzyme and Microbial Technology* **38**(1–2): 273-278.

HUANG, J.-C. & LI, L. (2000). An innovative approach to maximize primary treatment performance. *Water Science and Technology* **42**(12): 209–222.

HUBBELL, S. P. (2001). *The unified neutral theory of biodiversity and biogeography*. Princeton University Press, Princeton, NJ, U.S.A. 448 pp.

HVITVED-JACOBSEN, T., VOLLERTSEN, J. & NIELSEN, P. H. (1998). A process and model concept for microbial wastewater transformations in gravity sewers. *Water Science and Technology* **37**(1): 233-241.

IBARBALZ, F. M., PEREZ, M. V., FIGUEROLA, E. L. & ERIJMAN, L. (2014). The bias associated with amplicon sequencing does not affect the quantitative assessment of bacterial community dynamics. *PLoS ONE* **9**(6): e99722.

IMHOFF, K. (1955). Two-stage operation of activated sludge plants. *Sewage and Industrial Wastes* **27**(4): 431-433.

JENICEK, P., KUTIL, J., BENES, O., TODT, V., ZABRANSKA, J. & DOHANYOS, M. (2013). Energy self-sufficient sewage wastewater treatment plants: Is optimized anaerobic sludge digestion the key? *Water Science and Technology* **68**(8): 1739-1744.

JENKINS, D., RICHARD, M. G. & DAIGGER, G. T. (2004). *Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems*, 3rd edition. CRC Press LLC, London, England.

JENNI, S., VLAEMINCK, S. E., MORGENROTH, E. & UDERT, K. M. (2014). Successful application of nitritation/anammox to wastewater with elevated organic carbon to ammonia ratios. *Water Research* **49**: 316-326.

JIANG, F., BECK, M. B., CUMMINGS, R. G., ROWLES, K. & RUSSELL, D. (2005). Estimation of costs of phosphorus removal in wastewater treatment facilities: Adaptation of existing facilities. *Water Policy Working Paper #2005-011*.

JIMENEZ, J., MILLER, M., BOTT, C., MURTHY, S., DE CLIPPELEIR, H. & WETT, B. (2015). High-rate activated sludge system for carbon management – Evaluation of crucial process mechanisms and design parameters. *Water Research* **87**: 476-482.

JIMENEZ, J. A., LA MOTTA, E. J. & PARKER, D. S. (2007). Effect of operational parameters on the removal of particulate chemical oxygen demand in the activated sludge process. *Water Environment Research* **79**(9): 984-990.

JOHNSON, D. R., LEE, T. K., PARK, J., FENNER, K. & HELBLING, D. E. (2014). The functional and taxonomic richness of wastewater treatment plant microbial communities are associated with each other and with ambient nitrogen and carbon availability. *Environmental Microbiology*.

JU, F., XIA, Y., GUO, F., WANG, Z. & ZHANG, T. (2014). Taxonomic relatedness shapes bacterial assembly in activated sludge of globally distributed wastewater treatment plants. *Environmental Microbiology* **16**(8): 2421-2432.

JU, F. & ZHANG, T. (2014). Bacterial assembly and temporal dynamics in activated sludge of a full-scale municipal wastewater treatment plant. *The ISME journal*: 1-13.

JUDD, S. & JUDD, C. (EDS). (2006). *The MBR Book: Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment*. Elsevier Science, Oxford, UK.

JURETSCHKO, S., TIMMERMANN, G., SCHMID, M., SCHLEIFER, K.-H., POMMERENING-RÖSER, A., KOOPS, H.-P. & WAGNER, M. (1998). Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. *Applied and Environmental Microbiology* **64**(8): 3042-3051.

KAEWPIPAT, K. & GRADY, C. P., JR. (2002). Microbial population dynamics in laboratory-scale activated sludge reactors. *Water Science and Technology* **46**(1-2): 19-27.

KALMBACH, S., MANZ, W., WECKE, J. & SZEWZYK, U. (1999). *Aquabacterium* gen. nov., with description of *Aquabacterium citratiphilum* sp. nov., *Aquabacterium parvum* sp. nov. and *Aquabacterium commune* sp. nov., three in situ dominant bacterial species from the Berlin drinking water system. *International Journal of Systematic Bacteriology* **49 Pt 2**: 769-77.

KARAHAN-GÜL, Ö., ARTAN, N., ORHON, D., HENZE, M. & VAN LOOSDRECHT, M. C. M. (2002). Respirometric assessment of storage yield for different substrates. *Water Science and Technology* **46**(1-2): 345-352.

KARAPINAR, N. (2009). Application of natural zeolite for phosphorus and ammonium removal from aqueous solutions. *Journal of Hazardous Materials* **170**(2–3): 1186-1191.

KARR, D. B., WATERS, J. K. & EMERICH, D. W. (1983). Analysis of Poly-beta-Hydroxybutyrate in *Rhizobium japonicum* Bacteroids by Ion-Exclusion High-Pressure Liquid Chromatography and UV Detection. *Applied and Environmental Microbiology* **46**(6): 1339-1344.

KARTAL, B., KUENEN, J. G. & VAN LOOSDRECHT, M. C. (2010). Sewage treatment with anammox. *Science* **328**(5979): 702-703.

KHANAL, S. K. (2008). Overview of anaerobic biotechnology. In *Anaerobic Biotechnology for Bioenergy Production* (ed. S. K. Khanal), pp. 1-27. Wiley-Blackwell, Hoboken, NJ, U.S.A.

KHARARJIAN, H. A. & SHERRARD, J. H. (1977). Batch aerobic treatment of a colloidal wastewater. *Journal - Water Pollution Control Federation* **49**(9): 1985-1992.

KHARARJIAN, H. A. & SHERRARD, J. H. (1978). Contact stabilization treatment of a colloidal organic wastewater. *Journal - Water Pollution Control Federation* **50**(4): 645-652.

KIM, T. S., JEONG, J. Y., WELLS, G. F. & PARK, H. D. (2013). General and rare bacterial taxa demonstrating different temporal dynamic patterns in an activated sludge bioreactor. *Applied Microbiology and Biotechnology* **97**(4): 1755-1765.

KOCH, R. (1882). Die Ätiologie der Tuberkulose. *Berliner Klinische Wochenschrift* **19**: 221-230.

KOJIMA, H. & FUKUI, M. (2011). *Sulfuritalea hydrogenivorans* gen. nov., sp. nov., a facultative autotroph isolated from a freshwater lake. *International Journal of Systematic and Evolutionary Microbiology* **61**(Pt 7): 1651-1655.

KOS, M., WANNER, J., SORM, I. & GRAU, P. (1992). R-D-N activated sludge process. *Water Science and Technology* **25**(4-5): 151-160.

KRAUS, L. S. (1945). The use of digested sludge and digester overflow to control bulking activated sludge. *Sewage Works Journal* **17**(6): 1177-1190.

LACKNER, S., GILBERT, E. M., VLAEMINCK, S. E., JOSS, A., HORN, H. & VAN LOOSDRECHT, M. C. M. (2014). Full-scale partial nitritation/anammox experiences – An application survey. *Water Research* **55**: 292-303.

LARREA, L., IRIZAR, I. & HIDALGO, M. E. (2002). Improving the predictions of ASM2d through modelling in practice. *Water Science and Technology* **45**(6): 199-208.

LAWRENCE, A. W. & MCCARTY, P. L. (1970). Unified basis for biological treatment design and operation. *Journal of the Sanitary Engineering Division* **96**(3): 757-778.

LEE, J. Z., LOGAN, A., TERRY, S. & SPEAR, J. R. (2015). Microbial response to single-cell protein production and brewery wastewater treatment. *Microbial Biotechnology* **8**(1): 65-76.

LEE, W. S., CHUA, A. S. M., YEOH, H. K. & NGOH, G. C. (2014). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal* **235**: 83-99.

LEHMANN, J. (2007). A handful of carbon. *Nature* **447**(7141): 143-144.

LENNON, J. T. & JONES, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Micro* **9**(2): 119-130.

LI, X. Y. & YANG, S. F. (2007). Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research* **41**(5): 1022-1030.

LIAO, B. Q., ALLEN, D. G., DROPPO, I. G., LEPPARD, G. G. & LISS, S. N. (2001). Surface properties of sludge and their role in bioflocculation and settleability. *Water Research* **35**(2): 339-350.

LIAO, B. Q., DROPPO, I. G., LEPPARD, G. G. & LISS, S. N. (2006). Effect of solids retention time on structure and characteristics of sludge flocs in sequencing batch reactors. *Water Research* **40**(13): 2583-2591.

LIBRALATO, G., VOLPI GHIRARDINI, A. & AVEZZÙ, F. (2012). To centralise or to decentralise: An overview of the most recent trends in wastewater treatment management. *Journal of Environmental Management* **94**(1): 61-68.

LIU, R., WU, P., LANG, L., XU, C. & WANG, Y. (2016). Free Fe3+/Fe2+ improved the biomass resource recovery and organic matter removal in *Rhodobacter sphaeroides* purification of sewage. *Environmental Technology* **37**(1): 108-113.

LIU, S.-G., NI, B.-J., WEL, L., TANG, Y. & YU, H.-Q. (2009). Contact-adsorption-regeneration-stabilization process for the treatment of municipal wastewater. *Journal of Water and Environment Technology* **7**(2): 83-90.

LIU, X.-M., SHENG, G.-P., LUO, H.-W., ZHANG, F., YUAN, S.-J., XU, J., ZENG, R. J., WU, J.-G. & YU, H.-Q. (2010). Contribution of extracellular polymeric substances (EPS) to the sludge aggregation. *Environmental Science & Technology* **44**(11): 4355-4360.

LIU, Y., WANG, Z.-W., QIN, L., LIU, Y.-Q. & TAY, J.-H. (2005). Selection pressure-driven aerobic granulation in a sequencing batch reactor. *Applied Microbiology and Biotechnology* **67**(1): 26-32.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**(1): 265-75.

LUDZACK, F. J. & ETTINGER, M. B. (1962). Controlling operation to minimize activated sludge effluent nitrogen. *Journal - Water Pollution Control Federation* **34**(9): 920-931.

LYNCH, M. D. J. & NEUFELD, J. D. (2015). Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology* **13**(4): 217-229.

MAGURRAN, A. E. & HENDERSON, P. A. (2003). Explaining the excess of rare species in natural species abundance distributions. *Nature* **422**(6933): 714-716.

MAJONE, M., DIRCKS, K. & BEUN, J. J. (1999). Aerobic storage under dynamic conditions in activated sludge processes. The state of the art. *Water Science and Technology* **39**(1): 61-73.

MAKINIA, J., ROSENWINKEL, K. H. & PHAN, L. C. (2006). Modification of ASM3 for the determination of biomass adsorption/storage capacity in bulking sludge control. *Water Science and Technology* **53**(3): 91-99.

MAMAIS, D., JENKINS, D. & PRRR, P. (1993). A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Research* **27**(1): 195-197.

MANCELL-EGALA, A., KINNEAR, D., MURTHY, S. & JONES, K. (2012). Settling transition concentration measurement to quantify sludge settling behavior. In *Proceedings of the 86th Water Environment Federation Technical Exhibition and Conference (WEFTEC)*, pp. 5735-5746, 29 September - 3 October 2012, New Orleans, LA, U.S.A.

MANCELL-EGALA, W. A., KINNEAR, D. J., JONES, K. L., DE CLIPPELEIR, H., TAKACS, I. & MURTHY, S. N. (2016). Limit of stokesian settling concentration characterizes sludge settling velocity. *Water Research* **90**: 100-110.

MARTÍNEZ I PUENTES, M. (2006). A dynamic knowledge-based decision support system to handle solids separation problems in activated sludge systems: Development and validation. PhD thesis, University of Girona.

MARTINS, A. M., HEIJNEN, J. J. & VAN LOOSDRECHT, M. C. (2003a). Effect of dissolved oxygen concentration on sludge settleability. *Applied Microbiology and Biotechnology* **62**(5-6): 586-593.

MARTINS, A. M. P., HEIJNEN, J. J. & VAN LOOSDRECHT, M. C. M. (2003b). Effect of feeding pattern and storage on the sludge settleability under aerobic conditions. *Water Research* **37**(11): 2555-2570.

MARTINS, A. M. P., KARAHAN, Ö. & VAN LOOSDRECHT, M. C. M. (2011). Effect of polymeric substrate on sludge settleability. *Water Research* **45**(1): 263-273.

MATASSA, S., BOON, N. & VERSTRAETE, W. (2015). Resource recovery from used water: The manufacturing abilities of hydrogen-oxidizing bacteria. *Water Research* **68**(0): 467-478.

MATĚJŮ, V., ČIŽINSKÁ, S., KREJČÍ, J. & JANOCH, T. (1992). Biological water denitrification - A review. *Enzyme and Microbial Technology* **14**(3): 170-183.

MATSCHÉ, N. & MOSER, D. (1993). Operation of a two-stage activated sludge package plant for high efficiency treatment. *Water Science and Technology* **28**(10): 299-307.

MATSCHÉ, N. & WINKLER, S. (2014). The 2-stage hybrid approach for AS-plants: Plant adaptation with minimal space requirements. Poster presentation. In *Activated Sludge - 100 Years and Counting*, 12 - 14 June 2014, Essen, Germany.

MAURER, M., ROTHENBERGER, D. & LARSEN, T. A. (2005). Decentralised wastewater treatment technologies from a national perspective: At what cost are they competitive? *Water Science and Technology: Water Supply* **5**(6): 145-154.

MCILROY, S. J., SAUNDERS, A. M., ALBERTSEN, M., NIERYCHLO, M., MCILROY, B., HANSEN, A. A., KARST, S. M., NIELSEN, J. L. & NIELSEN, P. H. (2015). MiDAS: The field guide to the microbes of activated sludge (Available at http://www.midasfieldguide.org/).

McKinney, R. E. (2004). *Environmental pollution control microbiology: A fifty-year perspective*. Marcel Dekker, New York, U.S.A.

McMurdie, P. J. & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**(4): e61217.

Meerburg, F. A., Boon, N., Van Winckel, T., Vercamer, J. A. R., Nopens, I. & Vlaeminck, S. E. (2015). Toward energy-neutral wastewater treatment: A high-rate contact stabilization process to maximally recover sewage organics. *Bioresource Technology* **179**: 373-381.

Menkveld, H. W. H. & Broeders, E. (2015). Recovery of ammonium from digestate as fertilizer. In *1st IWA Resource Recovery Conference*, 30 August - 2 September, Ghent, Belgium.

Menniti, A., Kang, S., Elimelech, M. & Morgenroth, E. (2009). Influence of shear on the production of extracellular polymeric substances in membrane bioreactors. *Water Research* **43**(17): 4305-4315.

Metcalf & Eddy. (2003). *Wastewater Engineering: Treatment and Reuse*. 4th edition, McGraw-Hill, New York, U.S.A.

Methanex. (2016). Methanex posts regional contract methanol prices for North America, Europe and Asia. (Available at https://www.methanex.com/our-business/pricing). (Accessed 27 May 2016).

Mo, W. & Zhang, Q. (2012). Can municipal wastewater treatment systems be carbon neutral? *Journal of Environmental Management* **112**: 360-367.

Modin, O., Gustavsson, D. J. I., Tumlin, S., Mattsson, A., Jansen, J. L. C. & Wilén, B.-M. (2014). Back to basics: High-loaded activated sludge. In *Activated Sludge - 100 Years and Counting*, 12 - 14 June 2014, Essen, Germany.

Monod, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology* **3**(1): 371-394.

More, T. T., Yadav, J. S., Yan, S., Tyagi, R. D. & Surampalli, R. Y. (2014). Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Environmental Management* **144**: 1-25.

Morgan-Sagastume, F., Hjort, M., Cirne, D., Gerardin, F., Lacroix, S., Gaval, G., Karabegovic, L., Alexandersson, T., Johansson, P., Karlsson, A., Bengtsson, S., Arcos-Hernandez, M. V., Magnusson, P. & Werker, A. (2015). Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresource Technology* **181C**: 78-89.

Morgan, J. W., Forster, C. F. & Evison, L. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research* **24**(6): 743-750.

Mulder, A. (2003). The quest for sustainable nitrogen removal technologies. *Water Science and Technology* **48**(1): 67-75.

Mulkerrins, D., Dobson, A. D. W. & Colleran, E. (2004). Parameters affecting biological phosphate removal from wastewaters. *Environment International* **30**(2): 249-259.

Müller, E. A. & Kobel, B. (2004). Stocktaking at wastewater treatment plants in North Rhine Westphalia with 30 million population equivalents – Energy benchmarking and savings potentials (*in German*). *KA Korrespondenz Abwasser, Abfall*.

Nansubuga, I., Banadda, N., Ronsse, F., Verstraete, W. & Rabaey, K. (2015). Digestion of high rate activated sludge coupled to biochar formation for soil improvement in the tropics. *Water Research* **81**: 216-222.

Ng, H. Y. & Hermanowicz, S. W. (2005). Membrane bioreactor operation at short solids retention times: performance and biomass characteristics. *Water Research* **39**(6): 981-992.

Ni, B.-J., Yu, H.-Q. & Sun, Y.-J. (2008). Modeling simultaneous autotrophic and heterotrophic growth in aerobic granules. *Water Research* **42**(6–7): 1583-1594.

Ni, B.-J., Yu, H.-Q. & Zeng, R. J. (2014). Understanding the microbial internal storage turnover in wastewater treatment: Retrospect, prospect, and challenge. *Critical Reviews in Environmental Science and Technology* **45**(6): 591-612.

NOGAJ, T., RANDALL, A., JIMENEZ, J., TAKACS, I., BOTT, C., MILLER, M., MURTHY, S. & WETT, B. (2015). Modeling of organic substrate transformation in the high-rate activated sludge process. *Water Science and Technology* **71**(7): 971-979.

NOPENS, I. (2005). Modelling the activated sludge flocculation process: A population balance approach. PhD thesis, Faculty of Bioscience Engineering, Ghent University.

NOWAK, O., KEIL, S. & FIMML, C. (2011). Examples of energy self-sufficient municipal nutrient removal plants. *Water Science and Technology* **64**(1): 1-6.

OFIŢERU, I. D., LUNN, M., CURTIS, T. P., WELLS, G. F., CRIDDLE, C. S., FRANCIS, C. A. & SLOAN, W. T. (2010). Combined niche and neutral effects in a microbial wastewater treatment community. *Proceedings of the National Academy of Sciences of the United States of America* **107**(35): 15345-15350.

OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R. B., SIMPSON, G. L., SOLYMOS, P., STEVENS, M. H. H. & WAGNER, H. (2013). Vegan: Community ecology package. *R package version 2.0-9.* (Available at http://CRAN.R-project.org/package=vegan).

ORHON, D. (2015). Evolution of the activated sludge process: The first 50 years. *Journal of Chemical Technology & Biotechnology* **90**(4): 608-640.

OSHIKI, M., ONUKI, M., SATOH, H. & MINO, T. (2010). Separation of PHA-accumulating cells in activated sludge based on differences in buoyant density. *The Journal of General and Applied Microbiology* **56**(2): 163-167.

PAINE, R. T. (1995). A Conversation on Refining the Concept of Keystone Species. *Conservation Biology* **9**(4): 962-964.

PALMER, S. & NAIR, A. (2011). The developing value of sludge and how to exploit it. In *16th European Biosolids and Organic Resources Conference*, 13 - 15 November 2011, Leeds, UK.

PAUL, E., CAMACHO, P., SPERANDIO, M. & GINESTET, P. (2006). Technical and economical evaluation of a thermal, and two oxidative techniques for the reduction of excess sludge production. *Process Safety and Environmental Protection* **84**(4): 247-252.

PEDROS-ALIO, C. (2012). The rare bacterial biosphere. *Annual Review of Marine Science* **4**: 449-466.

PETRIE, B., MCADAM, E. J., LESTER, J. N. & CARTMELL, E. (2014). Assessing potential modifications to the activated sludge process to improve simultaneous removal of a diverse range of micropollutants. *Water Research* **62**: 180-192.

POLIZZI, C. (2013). Dynamic evaluation of a high loaded activated sludge plant as pretreatment for deammonification in the mainstream. Master thesis, University of Padova.

PRESTON, F. W. (1960). Time and space and the variation of species. *Ecology* **41**(4): 612-627.

R CORE TEAM. (2013). R: A language and environment for statistical computing. http://www.R-project.org/.

RACHID, S., GERTH, K., KOCHEMS, I. & MÜLLER, R. (2007). Deciphering regulatory mechanisms for secondary metabolite production in the myxobacterium *Sorangium cellulosum* So ce56. *Molecular Microbiology* **63**(6): 1783-1796.

RAHMAN, A., CLIPPELEIR, H. D., WINCKEL, T. V., LE, T., RIFFAT, R., WETT, B., JIMENEZ, J. A., BOTT, C., AL-OMARI, A. & MURTHY, S. (2015). Does optimization of carbon redirection always imply energy recovery? In *Proceedings of the 88th Water Environment Federation Technical Exhibition and Conference (WEFTEC)*.

RAHMAN, A., MEERBURG, F. A., RAVADAGUNDHI, S., WETT, B., JIMENEZ, J., BOTT, C., AL-OMARI, A., RIFFAT, R., MURTHY, S. & DE CLIPPELEIR, H. (2016). Bioflocculation management through high-rate contact-stabilization: A promising technology to recover organic carbon from low-strength wastewater. *Water Research* **104**: 485-496.

RAMALHO, R. S. (1977a). Chapter 5: Secondary treatment: The activated sludge process. In *Introduction to Wastewater Treatment Processes*. Academic Press, New York, U.S.A.

RAMALHO, R. S. (1977b). Chapter 6: Secondary treatment: Other aerobic and anaerobic wastewater treatment processes. In *Introduction to Wastewater Treatment Processes*. Academic Press, New York, U.S.A.

RAMETTE, A. (2007). Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**(2): 142-160.

RITTMANN, B. (1996). How Input Active Biomass Affects Sludge Age and Process Stability. *Journal of Environmental Engineering* **122**(1): 4-8.

RITTMANN, B. E. & MCCARTY, P. L. (2001). Chapter 6: The activated sludge process. In *Environmental Biotechnology: Principles and applications*. Biological Science Series, McGraw-Hill International Editions, New York, U.S.A.

ROEST, K., DAAMEN, B., GRAAFF, M. D., HARTOG, L., ZANDVOORT, M., UIJTERLINDE, C., DILVEN, S., LIER, J. V. & LOOSDRECHT, M. V. (2012). Energy production from wastewater - Dynamic filtration of A-stage sludge. In *IWA World Congress on Water, Climate & Energy*, 13 - 18 May 2012, Dublin, Ireland.

RÖLING, W. F. M. & VAN BODEGOM, P. M. (2014). Toward quantitative understanding on microbial community structure and functioning: A modeling-centered approach using degradation of marine oil spills as example. *Frontiers in Microbiology* **5**: 125.

ROSSELLÓ-MORA, R. A., WAGNER, M., AMANN, R. & SCHLEIFER, K. H. (1995). The abundance of *Zoogloea ramigera* in sewage treatment plants. *Applied and Environmental Microbiology* **61**(2): 702-707.

ROTTHAUWE, J. H., WITZEL, K. P. & LIESACK, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology* **63**(12): 4704-4712.

ROUME, H., HEINTZ-BUSCHART, A., MULLER, E. E. L., MAY, P., SATAGOPAM, V. P., LACZNY, C. C., NARAYANASAMY, S., LEBRUN, L. A., HOOPMANN, M. R., SCHUPP, J. M., GILLECE, J. D., HICKS, N. D., ENGELTHALER, D. M., SAUTER, T., KEIM, P. S., MORITZ, R. L. & WILMES, P. (2015). Comparative integrated omics: identification of key functionalities in microbial community-wide metabolic networks. *Npj Biofilms And Microbiomes* **1**: 15007.

RYBICKI, S. (1997). Advanced Wastewater Treatment Report no.1: Phosphorus removal from wastewater. A literature review. (ed. E. Płaza, E. Levlin and B. Hultman). Division of Water Resources Engineering, Royal Institute of Technology, Stockholm.

SAIKALY, P. E. & OERTHER, D. B. (2004). Bacterial competition in activated sludge: Theoretical analysis of varying solids retention times on diversity. *Microbial Ecology* **48**(2): 274-284.

SAIKALY, P. E. & OERTHER, D. B. (2011). Diversity of dominant bacterial taxa in activated sludge promotes functional resistance following toxic shock loading. *Microbial Ecology* **61**(3): 557-567.

SAIKALY, P. E., STROOT, P. G. & OERTHER, D. B. (2005). Use of 16S rRNA gene terminal restriction fragment analysis to assess the impact of solids retention time on the bacterial diversity of activated sludge. *Applied and Environmental Microbiology* **71**(10): 5814–5822.

SALOMÉ, A. A. (1990). AB-systemen: Een inventarisatie (*in Dutch*). In *Report RWZI 2000 90-02*, pp. 63. DBW/RIZA & STORA. Available at: http://www.stowa.nl/bibliotheek/publicaties/AB-systemen__een_inventarisatie_.

SARIOGLU, M., ORHON, D., GORGUN, E. & ARTAN, N. (2003). Design procedure for carbon removal in contact stabilization activated sludge process. *Water Science and Technology* **48**(11-12): 285-292.

SAUNDERS, A. M., ALBERTSEN, M., VOLLERTSEN, J. & NIELSEN, P. H. (2016). The activated sludge ecosystem contains a core community of abundant organisms. *The ISME Journal* **10**(1): 11-20.

SCHAUBROECK, T., DE CLIPPELEIR, H., WEISSENBACHER, N., DEWULF, J., BOECKX, P., VLAEMINCK, S. E. & WETT, B. (2015). Environmental sustainability of an energy self-sufficient sewage treatment plant: Improvements through DEMON and co-digestion. *Water Research* **74**(0): 166-179.

SCHERSON, Y. D. & CRIDDLE, C. S. (2014). Recovery of freshwater from wastewater: Upgrading process configurations to maximize energy recovery and minimize residuals. *Environmental Science & Technology* **48**(15): 8420-8432.

SCHLOSS, P. D., WESTCOTT, S. L., RYABIN, T., HALL, J. R., HARTMANN, M., HOLLISTER, E. B., LESNIEWSKI, R. A., OAKLEY, B. B., PARKS, D. H., ROBINSON, C. J., SAHL, J. W., STRES, B., THALLINGER, G. G., VAN HORN, D. J.

& WEBER, C. F. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**(23): 7537-7541.

SCHULER, A. I. & JANG, H. (2007). Causes of variable biomass density and its effects on settleability in full-scale biological wastewater treatment systems. *Environmental Science & Technology* **41**(5): 1675-1681.

SCHULZE-RETTMER, R., KIM, S. S. & S., S. S. (1992). Experience with two-stage activated sludge plants for industrial wastewaters in Korea. *Water Science and Technology* **25**(4-5): 427-428.

SCHÜRMANN, B. (1984). Biocoenosen von Belebungsanlagen underschiedlicher Schlammbelastung (*in German*). In *A-B-Technologie: Erfahrungsaustausch*. Gesellschaft zur Förderung der Siedlungswasserwirtschaft, 26 - 28 September 1983, Aachen, Germany.

SEDLAK, D. (2014). *Water 4.0: The past, present, and future of the world's most vital resource*. Yale University Press, New Haven, U.S.A.

SEUNTJENS, D., BUNDERVOET, B. L. M., MOLLEN, H., DE MULDER, C., WYPKEMA, E., VERLIEFDE, A., NOPENS, I., COLSEN, J. G. M. & VLAEMINCK, S. E. (2016). Energy efficient treatment of A-stage effluent: Pilot-scale experiences with shortcut nitrogen removal. *Water Science and Technology*.

SHADE, A., CAPORASO, J. G., HANDELSMAN, J., KNIGHT, R. & FIERER, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *The ISME Journal* **7**(8): 1493-1506.

SHADE, A., JONES, S. E., CAPORASO, J. G., HANDELSMAN, J., KNIGHT, R., FIERER, N. & GILBERT, J. A. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio* **5**(4).

SHANNON, P., MARKIEL, A., OZIER, O., BALIGA, N. S., WANG, J. T., RAMAGE, D., AMIN, N., SCHWIKOWSKI, B. & IDEKER, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research* **13**(11): 2498-2504.

SHENG, G.-P., YU, H.-Q. & LI, X.-Y. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances* **28**(6): 882-894.

SHU, L., SCHNEIDER, P., JEGATHEESAN, V. & JOHNSON, J. (2006). An economic evaluation of phosphorus recovery as struvite from digester supernatant. *Bioresource Technology* **97**(17): 2211-2216.

SIEGRIST, H. (1996). Nitrogen removal from digester supernatant - Comparison of chemical and biological methods. *Water Science and Technology* **34**(1-2): 399-406.

SIEGRIST, H., SALZGEBER, D., EUGSTER, J. & JOSS, A. (2008). Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Science and Technology* **57**(3): 383-388.

SMITH, A. L., SKERLOS, S. J. & RASKIN, L. (2013). Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. *Water Research* **47**(4): 1655-1665.

SOBECK, D. C. & HIGGINS, M. J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water Research* **36**(3): 527-538.

SOGIN, M. L., MORRISON, H. G., HUBER, J. A., WELCH, D. M., HUSE, S. M., NEAL, P. R., ARRIETA, J. M. & HERNDL, G. J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America* **103**(32): 12115-12120.

SÖRME, L. & LAGERKVIST, R. (2002). Sources of heavy metals in urban wastewater in Stockholm. *Science of The Total Environment* **298**(1–3): 131-145.

SPÄTH, R., FLEMMING, H. C. & WUERTZ, S. (1998). Sorption properties of biofilms. *Water Science and Technology* **37**(4–5): 207-210.

STARKEY, J. E. & KARR, P. R. (1984). Effect of low dissolved oxygen concentration on effluent turbidity. *Journal - Water Pollution Control Federation* **56**(7): 837-843.

STROUS, M., HEIJNEN, J. J., KUENEN, G. J. & JETTEN, M. M. S. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* **50**(5): 589-596.

SUEZ ENVIRONMENT. (2014). In the Cannes basin, the wastewater treatment plant of the future is already here (Available at http://www.emag.suez-environnement.com/en/wp-content/uploads/2014/12/17-Aquaviva-VA.pdf). (Accessed 23 Sept. 2015).

SVANSTRÖM, M., FRÖLING, M., MODELL, M., PETERS, W. A. & TESTER, J. (2004). Environmental assessment of supercritical water oxidation of sewage sludge. *Resources, Conservation and Recycling* **41**(4): 321-338.

SWEETAPPLE, C., FU, G. & BUTLER, D. (2015). Does carbon reduction increase sustainability? A study in wastewater treatment. *Water Research*.

SYKES, R. M. (1975). Theoretical heterotrophic yields. *Journal - Water Pollution Control Federation* **47**(3): 591-600.

TAKÁCS, I., PATRY, G. G. & NOLASCO, D. (1991). A dynamic model of the clarification-thickening process. *Water Research* **25**(10): 1263-1271.

TAN, T. W., NG, H. Y. & ONG, S. L. (2008). Effect of mean cell residence time on the performance and microbial diversity of pre-denitrification submerged membrane bioreactors. *Chemosphere* **70**(3): 387-396.

TAY, J.-H., LIU, Q.-S. & LIU, Y. (2001). The effects of shear force on the formation, structure and metabolism of aerobic granules. *Applied Microbiology and Biotechnology* **57**(1): 227-233.

TAY, J.-H., PAN, S., TAY, S. T. L., IVANOV, V. & LIU, Y. (2003). The effect of organic loading rate on the aerobic granulation: The development of shear force theory. *Water Science and Technology* **47**(11): 235-240.

TEKSOY BAŞARAN, S., AYSEL, M., KURT, H., ERGAL, İ., AKARSUBAŞI, A., YAĞCI, N., DOĞRUEL, S., ÇOKGÖR, E. U., KESKINLER, B., SÖZEN, S. & ORHON, D. (2014). Kinetic characterization of acetate utilization and response of microbial population in super fast membrane bioreactor. *Journal of Membrane Science* **455**(0): 392-404.

TEKSOY BAŞARAN, S., AYSEL, M., KURT, H., ERGAL, İ., KUMRU, M., AKARSUBAŞI, A., SÖZEN, S. & ORHON, D. (2012). Removal of readily biodegradable substrate in super fast membrane bioreactor. *Journal of Membrane Science* **423–424**(0): 477-486.

TORFS, E. (2015). Different settling regimes in secondary settling tanks: Experimental process analysis, model development and calibration. PhD thesis, Department of Mathematical Modelling, Statistics and Bio-informatics, Universiteit Gent, Belgium.

TREVORS, J. T. (2011). Viable but non-culturable (VBNC) bacteria: Gene expression in planktonic and biofilm cells. *Journal of Microbiological Methods* **86**(2): 266-273.

TRZCINSKI, A. P., GANDA, L., KUNACHEVA, C., ZHANG, D. Q., LIN, L. L., TAO, G., LEE, Y. & NG, W. J. (2016a). Characterization and biodegradability of sludge from a high rate A-stage contact tank and B-stage membrane bioreactor of pilot-scale AB system treating municipal wastewaters. *Water Science and Technology*.

TRZCINSKI, A. P., GANDA, L., YAN NI, A. S., KUNACHEVA, C., QING, Z. D., LIN, L. L., TAO, G., LEE, Y. & NG, W. J. (2016b). Identification of recalcitrant compounds in a pilot-scale AB system: An adsorption (A) stage followed by a biological (B) stage to treat municipal wastewater. *Bioresource Technology* **206**: 121-127.

TSANG, Y. F., CHUA, H., SIN, S. N. & TAM, C. Y. (2006). A novel technology for bulking control in biological wastewater treatment plant for pulp and paper making industry. *Biochemical Engineering Journal* **32**(3): 127-134.

TSUSHIMA, I., KINDAICHI, T. & OKABE, S. (2007). Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. *Water Research* **41**(4): 785-794.

U.S. EPA. (2007). Opportunites for and benefits of combined heat and power at wastewater treatment facilities. EPA-430-R-07-003. *U.S. Environmental Protection Agency, Washington DC, 2007; p 42.*

U.S. EPA. (2014). Innovative wastewater treatment plant in Victorville, Calif. aims to go off-the-grid (Available at http://yosemite.epa.gov/opa/admpress.nsf/0/FF717AD88043ABE185257D5F005BD418). (Accessed 23 Sept. 2015).

ULRICH, W. & ZALEWSKI, M. (2006). Abundance and co-occurrence patterns of core and satellite species of ground beetles on small lake islands. *Oikos* **114**(2): 338-348.

UN. (2006). Water - A shared responsibility. United Nations World Water Development Report 2.

VALENTIN-VARGAS, A., TORO-LABRADOR, G. & MASSOL-DEYA, A. A. (2012). Bacterial community dynamics in full-scale activated sludge bioreactors: Operational and ecological factors driving community assembly and performance. *PLoS ONE* **7**(8): e42524.

VAN DER GAST, C. J., AGER, D. & LILLEY, A. K. (2008). Temporal scaling of bacterial taxa is influenced by both stochastic and deterministic ecological factors. *Environmental Microbiology* **10**(6): 1411-1418.

VAN DER GAST, C. J., JEFFERSON, B., REID, E., ROBINSON, T., BAILEY, M. J., JUDD, S. J. & THOMPSON, I. P. (2006). Bacterial diversity is determined by volume in membrane bioreactors. *Environmental Microbiology* **8**(6): 1048-1055.

VAN HAANDEL, A. C. & VAN DER LUBBE, J. (2007). *Handbook biological wastewater treatment - Design and optimisation of activated sludge systems*. 1st edition, Uitgeverij Quist, Leidschendam, the Netherlands.

VAN HOUTTE, E. & VERBAUWHEDE, J. (2012). Sustainable groundwater management using reclaimed water: the Torreele/St-André case in Flanders, Belgium. *Journal of Water Supply: Research and Technology - AQUA* **61**(8): 473-483.

VAN KESSEL, M. A. H. J., SPETH, D. R., ALBERTSEN, M., NIELSEN, P. H., OP DEN CAMP, H. J. M., KARTAL, B., JETTEN, M. S. M. & LÜCKER, S. (2015). Complete nitrification by a single microorganism. *Nature* **528**(7583): 555-559.

VAN LIER, J. B. & LETTINGA, G. (1999). Appropriate technologies for effective management of industrial and domestic waste waters: The decentralised approach. *Water Science and Technology* **40**(7): 171-183.

VAN LOOSDRECHT, M. C. M. & HENZE, M. (1999). Maintenance, endogeneous respiration, lysis, decay and predation. *Water Science and Technology* **39**(1): 107-117.

VAN LOOSDRECHT, M. C. M., POT, M. A. & HEIJNEN, J. J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Science and Technology* **35**(1): 41-47.

VÁSQUEZ-SARRIA, N., RODRÍGUEZ-VICTORIA, J. A., TORRES-LOZADA, P. & PARRA, C. A. M.-. (2011). Performance of a contact stabilization process for domestic wastewater treatment of Cali, Colombia. *Dyna-Colombia* **78**(168): 98-107.

VERSPRILLE, A. I., ZUURVEEN, B. & STEIN, T. (1985). The A-B process: A novel two stage wastewater treatment system. *Water Science and Technology* **17**(2-3): 235-246.

VERSTRAETE, W., VAN DE CAVEYE, P. & DIAMANTIS, V. (2009). Maximum use of resources present in domestic "used water". *Bioresource Technology* **100**(23): 5537-5545.

VERSTRAETE, W. & VLAEMINCK, S. E. (2011). ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. *International Journal of Sustainable Development and World Ecology* **18**(3): 253-264.

VILCHEZ-VARGAS, R., GEFFERS, R., SUÁREZ-DIEZ, M., CONTE, I., WALICZEK, A., KASER, V. S., KRALOVA, M., JUNCA, H. & PIEPER, D. H. (2013). Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system. *Environmental Microbiology* **15**(4): 1016-1039.

VLAEMINCK, S. E., TERADA, A., SMETS, B. F., DE CLIPPELEIR, H., SCHAUBROECK, T., BOLCA, S., DEMEESTERE, L., MAST, J., BOON, N., CARBALLA, M. & VERSTRAETE, W. (2010). Aggregate size and architecture determine microbial activity balance for one-stage partial nitritation and anammox. *Applied and Environmental Microbiology* **76**(3): 900-909.

WAHLBERG, E. J., KEINATH, T. M. & PARKER, D. S. (1994). Influence of activated sludge flocculation time on secondary clarification. *Water Environment Research* **66**(6): 779-786.

WAN, C., LEE, D.-J., YANG, X., WANG, Y., WANG, X. & LIU, X. (2015). Calcium precipitate induced aerobic granulation. *Bioresource Technology* **176**: 32-37.

WANDL, G. G., MÜLLER-RECHBERGER, H. H., MATSCHÉ, N. N., SVARDAL, K. K. & WINKLER, S. S. (2002). Two stage activated sludge plants - Influence of different operational modes on sludge bulking and nitrification. *Water Science and Technology* **46**(1-2): 479-486.

WANG, Q., GARRITY, G. M., TIEDJE, J. M. & COLE, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**(16): 5261-5267.

WBD. (2012). Bedrijfsresultaten zuiveringstechnische werken Waterschap Brabantse Delta (*in Dutch*). Waterschap Brabantse Delta, Breda, The Netherlands.

WEF. (2005). *Clarifier design. Water Environment Federation manual of practice no. FD-8.* . McGraw-Hill, New York, U.S.A.

WELLS, G. F., PARK, H. D., EGGLESTON, B., FRANCIS, C. A. & CRIDDLE, C. S. (2011). Fine-scale bacterial community dynamics and the taxa-time relationship within a full-scale activated sludge bioreactor. *Water Research* **45**(17): 5476-5488.

WENYI, D., HONG, D., LI-AN, Z., JIA, M. & BAOZHEN, W. (2006). Operational retrofits of AB process for biological removal of nitrogen and phosphorus. *Water Practice & Technology* **1**(4).

WETT, B., BUCHAUER, K. & FIMML, C. (2007). Energy self-sufficiency as a feasible concept for wastewater treatment systems. In *IWA Leading Edge Technology Conference*, pp. 21-24, September 2007, Singapore.

WETT, B., HELL, M., JIMENEZ, J., TAKACS, I., BOTT, C. & MURTHY, S. (2014). Operational and structural A-stage improvements for high-rate carbon removal. In *Global challenges: Sustainable Wastewater Treatment and Resource Recovery. IWA Specialist Conference*, 26 - 30 October 2014, Kathmandu, Nepal.

WIDIASTUTI, N., WU, H., ANG, H. M. & ZHANG, D. (2011). Removal of ammonium from greywater using natural zeolite. *Desalination* **277**(1–3): 15-23.

WILÉN, B.-M., JIN, B. & LANT, P. (2003). The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Research* **37**(9): 2127-2139.

WILÉN, B.-M., LUND NIELSEN, J., KEIDING, K. & NIELSEN, P. H. (2000). Influence of microbial activity on the stability of activated sludge flocs. *Colloids and Surfaces B: Biointerfaces* **18**(2): 145-156.

WILÉN, B. M., ONUKI, M., HERMANSSON, M., LUMLEY, D. & MINO, T. (2008). Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability. *Water Research* **42**(8-9): 2300-2308.

WILSENACH, J. & VAN LOOSDRECHT, M. (2006). Integration of processes to treat wastewater and source-separated urine. *Journal of Environmental Engineering* **132**(3): 331-341.

WINKLER, S., MATSCHE, N., GAMPERER, T. & DUM, M. (2004). Sewage-treatment under substantial load variations in winter tourism areas - A full case study. *Water Science and Technology* **50**(7): 147-155.

WITTEBOLLE, L., MARZORATI, M., CLEMENT, L., BALLOI, A., DAFFONCHIO, D., HEYLEN, K., DE VOS, P., VERSTRAETE, W. & BOON, N. (2009). Initial community evenness favours functionality under selective stress. *Nature* **458**(7238): 623-626.

WUHRMAN, K. (1960). Effects of oxygen tension on biochemical reactions in biological treatment plants. In *Advances in Biological Waste Treatment. Proceedings of the 3rd Conference at Manhattan College*, vol. 27, April 1960, New York City, U.S.A.

WUHRMANN, K. (1954). High-rate activated sludge treatment and its relation to stream sanitation: I. Pilot-plant studies. *Sewage and Industrial Wastes* **26**(1): 1-27.

XIE, B., GU, J. & LU, J. (2010). Surface properties of bacteria from activated sludge in relation to bioflocculation. *Journal of Environmental Sciences* **22**(12): 1840–1845.

XYLEM. (2012). Aeration products for energy-efficient biological wastewater treatment (Available at http://www.xylem.com/Assets/Resources/460-Sanitaire-Difussers.Jetaerators.Blowers-Aeration.pdf). (Accessed 6 June 2016).

YANG, S.-F. & LI, X.-Y. (2009). Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions. *Process Biochemistry* **44**(1): 91-96.

YU, K. & ZHANG, T. (2012). Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. *PLoS ONE* **7**(5): e38183.

YURKOVICH, J. T. & PALSSON, B. O. (2016). Solving puzzles with missing pieces: The power of systems biology. *Proceedings of the IEEE* **104**(1): 2-7.

ZESSNER, M., LAMPERT, C., KROISS, H. & LINDTNER, S. (2010). Cost comparison of wastewater treatment in Danubian countries. *Water Science and Technology* **62**(2): 223-230.

ZHANG, P., SHEN, Y., GUO, J. S., LI, C., WANG, H., CHEN, Y. P., YAN, P., YANG, J. X. & FANG, F. (2015a). Extracellular protein analysis of activated sludge and their functions in wastewater treatment plant by shotgun proteomics. *Scientific Reports* **5**: 12041.

ZHANG, X., LI, X., ZHANG, Q., PENG, Q., ZHANG, W. & GAO, F. (2014). New insight into the biological treatment by activated sludge: The role of adsorption process. *Bioresource Technology* **153**(0): 160-164.

ZHANG, X., ZHANG, H., YE, C., WEI, M. & DU, J. (2015b). Effect of COD/N ratio on nitrogen removal and microbial communities of CANON process in membrane bioreactors. *Bioresource Technology* **189**: 302-308.

ZHANG, Y. & ALLEN, D. G. (2008). The effect of short-term dissolved oxygen transients on activated sludge. *Water Quality Research Journal of Canada* **43**(2/3): 93-102.

ZHAO, W., TING, Y. P., CHEN, J. P., XING, C. H. & SHI, S. Q. (2000). Advanced primary treatment of waste water using a bio-flocculation-adsorption sedimentation process. *Acta Biotechnologica* **20**(1): 53-64.

ANNEX I:

SUPPLEMENTARY INFORMATION FOR CHAPTER 2

Table A.I.1

Network characteristics of phylotypes from the continuously abundant and transitional sub-communities of the **high-rate** system. Within each system, phylotypes are sorted on their "keystoneness" characteristics, as approximated by the sum of the betweenness centrality, (1 – closeness centrality) and node degree, each rescaled between 0 and 1.

High-rate community					
ID	Sub-community	Betweenness centrality	Closeness centrality	Node degree	Taxonomy
Phy 229	Transitional	0.019	0.247	35	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 208	Transitional	0.019	0.246	33	Bacteria: Bacteroidetes
Phy 313	Transitional	0.014	0.246	32	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 31	Transitional	0.143	0.263	30	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Rhodoferax
Phy 939	Transitional	0.004	0.238	28	Bacteria: Bacteroidetes: Bacteroidetes_incertae_sedis
Phy 7	Transitional	0.003	0.238	28	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae: Dokdonella
Phy 96	Transitional	0.010	0.240	27	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Hydrogenophaga
Phy 1508	Transitional	0.027	0.238	28	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Pseudacidovorax
Phy 42	Transitional	0.003	0.238	27	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Flammeovirgaceae: Fabibacter
Phy 241	Transitional	0.047	0.247	26	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Cystobacterineae: Cystobacteraceae: Anaeromyxobacter
Phy 714	Transitional	0.003	0.237	26	Bacteria: Bacteroidetes
Phy 880	Transitional	0.003	0.237	26	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae
Phy 540	Transitional	0.001	0.235	25	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales
Phy 829	Transitional	0.007	0.234	25	Bacteria: Bacteroidetes
Phy 299	Transitional	0.061	0.243	25	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Oxalobacteraceae
Phy 211	Transitional	0.036	0.242	24	Bacteria: Proteobacteria: Betaproteobacteria: Methylophilales: Methylophilaceae: Methylotenera
Phy 1332	Transitional	0.011	0.232	25	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 53	Transitional	0.001	0.234	24	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 368	Transitional	0.001	0.234	24	Bacteria: Proteobacteria: Alphaproteobacteria
Phy 749	Transitional	0.001	0.234	24	Bacteria: Bacteroidetes
Phy 386	Transitional	0.207	0.258	28	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Owenweeksia
Phy 26	Transitional	0.028	0.222	27	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 524	Transitional	0.001	0.234	23	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 1121	Transitional	0.001	0.234	23	Bacteria: Bacteroidetes: Bacteroidetes_incertae_sedis: Marinifilum
Phy 1282	Transitional	0.006	0.231	23	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 1046	Transitional	0.007	0.231	23	Bacteria: Proteobacteria: Gammaproteobacteria

Phy 757	Transitional	0.002	0.234	22	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 1664	Transitional	0.009	0.216	26	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 22	Transitional	0.010	0.242	20	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae
Phy 62	Transitional	0.094	0.228	24	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 51	Transitional	0.005	0.215	23	Bacteria: Fusobacteria: Fusobacteriia: Fusobacteriales
Phy 1585	Transitional	0.005	0.230	20	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 29	Transitional	0.008	0.214	23	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 207	Transitional	0.011	0.216	22	Bacteria: Proteobacteria
Phy 1607	Transitional	0.005	0.231	19	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales
Phy 232	Transitional	0.015	0.239	18	Bacteria: Proteobacteria: Deltaproteobacteria: Desulfuromonadales: Geobacteraceae: Geobacter
Phy 6	Transitional	0.026	0.238	18	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 525	Transitional	0.014	0.217	21	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 497	Transitional	0.012	0.233	18	Bacteria: Fusobacteria: Fusobacteriia: Fusobacteriales: Fusobacteriaceae: Propionigenium
Phy 1341	Transitional	0.000	0.218	20	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 333	Transitional	0.000	0.218	20	Bacteria: Proteobacteria: Deltaproteobacteria: Desulfovibrionales: Desulfomicrobiaceae: Desulfomicrobium
Phy 35	Transitional	0.008	0.237	17	Bacteria: Bacteroidetes
Phy 1467	Transitional	0.006	0.229	18	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales
Phy 46	Transitional	0.016	0.218	20	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 18	Continuoulsy abundant	0.053	0.242	17	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 11	Continuoulsy abundant	0.006	0.214	20	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 1283	Transitional	0.070	0.243	17	Bacteria: Proteobacteria: Betaproteobacteria
Phy 114	Transitional	0.028	0.227	18	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Thauera
Phy 482	Transitional	0.021	0.217	18	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 502	Transitional	0.013	0.202	20	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 263	Transitional	0.075	0.215	19	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
Phy 117	Transitional	0.019	0.225	15	Bacteria
Phy 914	Transitional	0.000	0.215	16	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Chryseobacterium
Phy 43	Transitional	0.137	0.258	14	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae: Leadbetterella
Phy 132	Transitional	0.064	0.216	17	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 686	Transitional	0.101	0.223	16	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Sulfurospirillum
Phy 859	Transitional	0.016	0.231	13	Bacteria
Phy 555	Transitional	0.000	0.209	15	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 8	Continuoulsy abundant	0.045	0.198	18	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 206	Transitional	0.000	0.231	12	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales
Phy 203	Transitional	0.008	0.201	16	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 1660	Transitional	0.008	0.201	16	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 95	Transitional	0.002	0.212	14	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 120	Transitional	0.017	0.207	15	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 1244	Transitional	0.038	0.245	11	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 1645	Transitional	0.013	0.199	16	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium

Phy 131	Transitional	0.006	0.196	16	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 271	Transitional	0.006	0.196	16	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 625	Transitional	0.008	0.201	15	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae
Phy 52	Transitional	0.019	0.215	13	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 996	Transitional	0.004	0.219	12	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Albidiferax
Phy 86	Transitional	0.103	0.225	13	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 239	Transitional	0.000	0.202	14	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Prevotellaceae: Prevotella
Phy 204	Transitional	0.000	0.208	13	Bacteria: Proteobacteria
Phy 334	Transitional	0.107	0.222	13	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 99	Transitional	0.012	0.235	10	Bacteria: Firmicutes: Clostridia: Clostridiales: Natranaerovirga
Phy 4	Continuoulsy abundant	0.046	0.197	15	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 1427	Transitional	0.020	0.234	10	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales
Phy 567	Transitional	0.000	0.218	11	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Rikenellaceae: Rikenella
Phy 192	Transitional	0.002	0.195	14	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 435	Transitional	0.006	0.229	10	Bacteria: Verrucomicrobia: Opitutae: Opitutales: Opitutaceae: Opitutus
Phy 565	Transitional	0.003	0.195	14	Bacteria: Firmicutes: Clostridia: Clostridiales: Clostridiales_Incertae Sedis XIII: Anaerovorax
Phy 367	Transitional	0.036	0.231	10	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 695	Transitional	0.004	0.205	12	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 139	Transitional	0.103	0.230	11	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 319	Transitional	0.006	0.196	13	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Rubrivivax
Phy 163	Transitional	0.006	0.195	13	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae
Phy 16	Transitional	0.027	0.197	13	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 433	Transitional	0.011	0.188	14	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 320	Transitional	0.000	0.216	10	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 249	Transitional	0.044	0.236	9	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Zoogloea
Phy 104	Transitional	0.000	0.198	12	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 461	Transitional	0.000	0.206	11	Bacteria
Phy 56	Transitional	0.005	0.195	12	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Chryseobacterium
Phy 761	Transitional	0.002	0.195	12	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 392	Transitional	0.036	0.228	9	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 317	Transitional	0.202	0.249	10	Bacteria: Firmicutes: Negativicutes: Selenomonadales: Veillonellaceae: Veillonella
Phy 338	Transitional	0.011	0.235	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 899	Transitional	0.086	0.250	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 349	Transitional	0.052	0.227	9	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
Phy 68	Transitional	0.081	0.232	9	Bacteria: Proteobacteria: Gammaproteobacteria: Methylococcales: Methylococcaceae: Methylococcus
Phy 801	Transitional	0.012	0.220	9	Bacteria: Proteobacteria: Epsilonproteobacteria
Phy 995	Transitional	0.000	0.198	11	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 900	Transitional	0.016	0.233	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Albidiferax
Phy 689	Transitional	0.021	0.231	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Rhodoferax
Phy 710	Transitional	0.035	0.234	8	Bacteria

Phy 276	Transitional	0.001	0.181	13	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 9	Continuoulsy abundant	0.147	0.215	11	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 671	Transitional	0.001	0.187	12	Bacteria: Firmicutes: Negativicutes: Selenomonadales: Veillonellaceae: Anaeromusa
Phy 1445	Transitional	0.001	0.187	12	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 1667	Transitional	0.001	0.187	12	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 812	Transitional	0.015	0.188	12	Bacteria
Phy 1039	Transitional	0.041	0.230	8	Bacteria: Proteobacteria: Gammaproteobacteria: Methylococcales: Methylococcaceae: Methylococcus
Phy 1208	Transitional	0.013	0.211	9	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae
Phy 409	Transitional	0.017	0.213	8	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae
Phy 372	Transitional	0.002	0.211	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 1762	Transitional	0.013	0.210	8	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 279	Transitional	0.000	0.187	10	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae
Phy 1436	Transitional	0.038	0.209	8	Bacteria: Verrucomicrobia: Spartobacteria: Spartobacteria_genera_incertae_sedis
Phy 106	Transitional	0.032	0.206	8	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Tolumonas
Phy 28	Continuoulsy abundant	0.110	0.218	8	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 17	Transitional	0.011	0.215	7	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 1378	Transitional	0.011	0.215	7	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Pseudacidovorax
Phy 519	Transitional	0.035	0.237	6	Bacteria: Bacteroidetes
Phy 506	Transitional	0.015	0.214	7	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 38	Continuoulsy abundant	0.017	0.214	7	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 402	Transitional	0.002	0.195	8	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Serpens
Phy 162	Transitional	0.008	0.194	8	Bacteria: Fusobacteria: Fusobacteriia: Fusobacteriales: Leptotrichiaceae
Phy 430	Transitional	0.034	0.210	7	Bacteria: Firmicutes: Clostridia: Clostridiales: Ruminococcaceae
Phy 69	Transitional	0.009	0.216	6	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Parabacteroides
Phy 181	Transitional	0.008	0.215	6	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Parabacteroides
Phy 343	Transitional	0.000	0.214	6	Bacteria: Firmicutes: Bacilli: Lactobacillales: Lactobacillaceae: Lactobacillus
Phy 603	Transitional	0.000	0.214	6	Bacteria: Actinobacteria: Actinobacteria: Actinobacteridae: Bifidobacteriales: Bifidobacteriaceae: Gardnerella
Phy 385	Transitional	0.009	0.185	8	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Prevotellaceae: Prevotella
Phy 1	Continuoulsy abundant	0.000	0.571	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Rhodoferax
Phy 1694	Transitional	0.000	0.571	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 361	Transitional	0.015	0.211	6	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Aquabacterium
Phy 1322	Transitional	0.033	0.234	5	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 25	Transitional	0.022	0.227	5	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Dechloromonas
Phy 737	Transitional	0.002	0.200	6	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
Phy 416	Transitional	0.002	0.198	6	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 485	Transitional	0.002	0.198	6	Bacteria
Phy 953	Transitional	0.003	0.198	6	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 76	Transitional	0.000	0.184	7	Bacteria: Proteobacteria: Betaproteobacteria: Methylophilales: Methylophilaceae: Methylotenera
Phy 347	Transitional	0.015	0.168	9	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Malikia
Phy 24	Transitional	0.000	0.195	6	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium

Phy 316	Transitional	0.001	0.194	6	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 55	Transitional	0.008	0.213	5	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 84	Continuoulsy abundant	0.008	0.213	5	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 79	Transitional	0.000	0.208	5	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Cryomorpha
Phy 1205	Transitional	0.000	0.167	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Macromonas
Phy 1056	Transitional	0.008	0.168	8	Bacteria: Verrucomicrobia: Spartobacteria: Spartobacteria_genera_incertae_sedis
Phy 293	Transitional	0.003	0.175	7	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Cloacibacterium
Phy 364	Transitional	0.048	0.208	5	Bacteria: Proteobacteria: Gammaproteobacteria: Enterobacteriales: Enterobacteriaceae
Phy 622	Transitional	0.011	0.202	5	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Aquabacterium
Phy 103	Transitional	0.028	0.228	4	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 67	Transitional	0.018	0.181	6	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
Phy 623	Transitional	0.153	0.215	5	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 160	Transitional	0.043	0.182	6	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Parabacteroides
Phy 906	Transitional	0.004	0.161	8	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae: Aquaspirillum
Phy 44	Transitional	0.005	0.165	7	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Ferribacterium
Phy 1621	Transitional	0.023	0.189	5	Bacteria: Firmicutes: Clostridia: Clostridiales: Lachnospiraceae
Phy 722	Transitional	0.009	0.205	4	Bacteria: Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae: Clostridium XI
Phy 716	Transitional	0.000	0.198	4	Bacteria: Proteobacteria: Alphaproteobacteria
Phy 357	Transitional	0.003	0.186	4	Bacteria: Proteobacteria: Gammaproteobacteria: Enterobacteriales: Enterobacteriaceae: Raoultella
Phy 71	Transitional	0.006	0.215	3	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 48	Continuoulsy abundant	0.004	0.212	3	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 1640	Transitional	0.000	0.178	4	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 233	Transitional	0.008	0.198	3	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 403	Transitional	0.008	0.196	3	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Parabacteroides
Phy 63	Transitional	0.011	0.196	3	Bacteria: Bacteroidetes
Phy 261	Transitional	0.000	0.191	3	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
Phy 251	Transitional	0.000	0.189	3	Bacteria: Proteobacteria
Phy 136	Transitional	0.000	0.188	3	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 50	Transitional	0.019	0.166	4	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Anaerorhabdus
Phy 706	Transitional	0.500	0.667	2	Bacteria: Fibrobacteres: Fibrobacteria: Fibrobacterales: Fibrobacteraceae: Fibrobacter
Phy 32	Transitional	0.000	0.146	6	Bacteria: Proteobacteria
Phy 156	Transitional	0.000	0.146	6	Bacteria: Verrucomicrobia: Spartobacteria: Spartobacteria_genera_incertae_sedis
Phy 355	Transitional	0.000	0.146	6	Bacteria: Proteobacteria: Deltaproteobacteria: Desulfuromonadales: Geobacteraceae: Geobacter
Phy 265	Transitional	0.031	0.187	3	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae
Phy 118	Transitional	0.000	0.183	3	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae
Phy 342	Transitional	0.000	0.179	3	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Sulfurospirillum
Phy 473	Transitional	0.000	0.176	3	Bacteria
Phy 494	Transitional	0.028	0.155	4	Bacteria: Bacteroidetes: Bacteroidetes_incertae_sedis: Marinifilum
Phy 152	Transitional	0.009	0.168	3	Bacteria: Firmicutes: Bacilli: Lactobacillales: Carnobacteriaceae: Trichococcus
Phy 15	Transitional	0.000	0.198	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Simplicispira

Phy 1630	Transitional	0.019	0.158	3	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Simplicispira
Phy 463	Transitional	0.000	0.193	2	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 382	Transitional	0.009	0.192	2	Bacteria: Proteobacteria: Epsilonproteobacteria
Phy 41	Transitional	0.000	0.190	2	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Uliginosibacterium
Phy 158	Transitional	0.002	0.187	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Comamonas
Phy 444	Transitional	0.000	0.176	2	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 27	Transitional	0.009	0.174	2	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 240	Transitional	0.010	0.135	4	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 817	Transitional	0.009	0.154	2	Bacteria: Firmicutes: Clostridia: Clostridiales: Clostridiales_Incertae Sedis XII: Fusibacter
Phy 70	Transitional	0.000	0.135	3	Bacteria: Bacteroidetes
Phy 527	Transitional	0.009	0.143	2	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 3	Continuoulsy abundant	0.000	0.137	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 1457	Transitional	0.000	0.137	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 315	Transitional	0.000	0.134	2	Bacteria: Bacteroidetes
Phy 198	Transitional	0.667	0.800	3	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Dechloromonas
Phy 94	Transitional	0.000	1.000	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 137	Transitional	0.000	1.000	1	Bacteria: Proteobacteria
Phy 147	Transitional	0.000	1.000	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 346	Transitional	0.000	1.000	1	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 311	Transitional	0.000	0.444	1	Bacteria: Proteobacteria
Phy 30	Transitional	0.000	0.190	1	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 144	Transitional	0.000	0.188	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 199	Transitional	0.000	0.186	1	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Dechloromonas
Phy 193	Transitional	0.000	0.179	1	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Cloacibacterium
Phy 983	Transitional	0.000	0.178	1	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Tolumonas
Phy 303	Transitional	0.000	0.175	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 436	Transitional	0.000	0.174	1	Bacteria: Firmicutes: Clostridia: Clostridiales: Lachnospiraceae
Phy 127	Transitional	0.000	0.170	1	Bacteria: Bacteroidetes
Phy 378	Transitional	0.000	0.168	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 331	Transitional	0.000	0.165	1	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae
Phy 440	Transitional	0.000	0.164	1	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 1028	Transitional	0.000	0.161	1	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae: Formivibrio
Phy 237	Transitional	0.000	0.156	1	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae
Phy 340	Transitional	0.000	0.148	1	Bacteria
Phy 576	Transitional	0.000	0.144	1	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae: Vitreoscilla
Phy 1429	Transitional	0.000	0.125	1	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae
Phy 231	Transitional	0.000	0.119	1	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Zoogloea

Table A.I.2

Network characteristics of phylotypes from the continuously abundant and transitional sub-communities of the **low-rate system**. Within each system, phylotypes are sorted on their "keystoneness" characteristics, as approximated by the sum of the betweenness centrality, (1 – closeness centrality) and node degree, each rescaled between 0 and 1.

Low-rate community

ID	Sub-community	Betweenness centrality	Closeness centrality	Node degree	Taxonomy
Phy 513	Transitional	0.015	0.274	86	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 542	Transitional	0.004	0.266	78	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 245	Transitional	0.004	0.265	74	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 559	Transitional	0.004	0.260	77	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 700	Transitional	0.003	0.264	68	Bacteria: Planctomycetes
Phy 563	Transitional	0.010	0.268	71	Bacteria
Phy 318	Transitional	0.002	0.262	67	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 324	Transitional	0.018	0.271	76	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Sinobacteraceae
Phy 518	Transitional	0.005	0.263	66	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Jahnella
Phy 850	Transitional	0.002	0.262	62	Bacteria
Phy 241	Transitional	0.012	0.270	65	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Cystobacterineae: Cystobacteraceae: Anaeromyxobacter
Phy 370	Transitional	0.016	0.269	69	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Verrucomicrobium
Phy 505	Transitional	0.011	0.267	66	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 7	Continuoulsy abundant	0.005	0.261	63	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae: Dokdonella
Phy 1049	Transitional	0.003	0.256	64	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 53	Transitional	0.013	0.273	63	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 448	Transitional	0.011	0.264	65	Bacteria
Phy 669	Transitional	0.002	0.261	59	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 787	Transitional	0.001	0.256	61	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 1170	Transitional	0.002	0.260	59	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 548	Transitional	0.001	0.253	59	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 574	Transitional	0.025	0.278	65	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 533	Transitional	0.016	0.277	57	Bacteria: Spirochaetes: Spirochaetia: Spirochaetales: Leptospiraceae: Turneriella
Phy 992	Transitional	0.001	0.253	57	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 256	Transitional	0.013	0.269	57	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 615	Transitional	0.001	0.253	57	Bacteria: Proteobacteria: Alphaproteobacteria

Phy 468	Transitional	0.014	0.266	59	Bacteria: Proteobacteria: Alphaproteobacteria
Phy 857	Transitional	0.001	0.252	56	Bacteria: Planctomycetes: Planctomycetia: Planctomycetales: Planctomycetaceae: Aquisphaera
Phy 232	Transitional	0.011	0.264	57	Bacteria: Proteobacteria: Deltaproteobacteria: Desulfuromonadales: Geobacteraceae: Geobacter
Phy 1267	Transitional	0.003	0.260	53	Bacteria: Latescibacteria: Latescibacteria_genera_incertae_sedis
Phy 258	Transitional	0.028	0.281	62	Bacteria: Bacteroidetes
Phy 1034	Transitional	0.003	0.260	52	Bacteria
Phy 875	Transitional	0.001	0.252	53	Bacteria
Phy 709	Transitional	0.000	0.251	52	Bacteria
Phy 938	Transitional	0.011	0.270	50	Bacteria: Proteobacteria
Phy 665	Transitional	0.005	0.266	48	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 865	Transitional	0.002	0.258	49	Bacteria
Phy 64	Transitional	0.014	0.255	58	Bacteria: Proteobacteria: Betaproteobacteria
Phy 1015	Transitional	0.001	0.251	51	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 645	Transitional	0.004	0.259	49	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 92	Transitional	0.020	0.261	58	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Aquabacterium
Phy 810	Transitional	0.002	0.258	47	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Flammeovirgaceae
Phy 91	Transitional	0.018	0.254	58	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 1439	Transitional	0.000	0.252	48	Bacteria: Planctomycetes: Planctomycetia: Planctomycetales: Planctomycetaceae
Phy 281	Transitional	0.010	0.253	53	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae
Phy 925	Transitional	0.004	0.255	48	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 628	Transitional	0.003	0.258	46	Bacteria
Phy 428	Transitional	0.003	0.264	44	Bacteria: Ignavibacteriae: Ignavibacteria: Ignavibacteriales: Ignavibacteriaceae: Ignavibacterium
Phy 680	Transitional	0.004	0.255	48	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 166	Transitional	0.005	0.250	50	Bacteria: Proteobacteria: Betaproteobacteria
Phy 1201	Transitional	0.002	0.250	48	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 445	Transitional	0.011	0.262	48	Bacteria: Bacteroidetes
Phy 273	Transitional	0.010	0.258	49	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 133	Transitional	0.008	0.266	45	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sulfuritalea
Phy 404	Transitional	0.005	0.260	45	Bacteria: Acidobacteria
Phy 958	Transitional	0.005	0.254	47	Bacteria
Phy 488	Transitional	0.007	0.246	52	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 501	Transitional	0.001	0.256	44	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 111	Transitional	0.004	0.241	52	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales
Phy 1495	Transitional	0.002	0.254	45	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 363	Transitional	0.006	0.247	50	Bacteria: Bacteroidetes
Phy 656	Transitional	0.004	0.257	45	Bacteria
Phy 235	Transitional	0.007	0.253	48	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae: Haliscomenobacter
Phy 220	Transitional	0.011	0.253	50	Bacteria: Bacteroidetes
Phy 103	Transitional	0.008	0.265	43	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 140	Transitional	0.006	0.245	49	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales

Phy 167	Transitional	0.012	0.256	47	Bacteria: Bacteroidetes: Bacteroidetes_incertae_sedis: Ohtaekwangia
Phy 126	Transitional	0.003	0.240	49	Bacteria: Proteobacteria
Phy 238	Transitional	0.001	0.257	41	Bacteria: Proteobacteria: Alphaproteobacteria: Caulobacterales: Hyphomonadaceae: Hirschia
Phy 332	Transitional	0.001	0.248	44	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 192	Transitional	0.005	0.248	46	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 8	Transitional	0.003	0.240	47	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 830	Transitional	0.009	0.249	46	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae
Phy 155	Transitional	0.004	0.240	47	Bacteria
Phy 1295	Transitional	0.003	0.250	42	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Terrimonas
Phy 145	Transitional	0.024	0.271	46	Bacteria: Acidobacteria: Acidobacteria_Gp4: Gp4
Phy 189	Transitional	0.029	0.255	57	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Terrimonas
Phy 119	Transitional	0.012	0.255	44	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae
Phy 353	Transitional	0.006	0.268	37	Bacteria
Phy 229	Transitional	0.000	0.244	42	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 276	Transitional	0.003	0.247	42	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 325	Transitional	0.004	0.250	41	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 729	Transitional	0.008	0.247	44	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 210	Transitional	0.008	0.250	43	Bacteria: Proteobacteria
Phy 756	Transitional	0.004	0.252	40	Bacteria: Verrucomicrobia: Subdivision3: Subdivision3_genera_incertae_sedis
Phy 453	Transitional	0.013	0.264	40	Bacteria: Bacteroidetes
Phy 1063	Transitional	0.007	0.267	36	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Ferruginibacter
Phy 731	Transitional	0.010	0.255	41	Bacteria
Phy 120	Transitional	0.006	0.249	41	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 146	Transitional	0.004	0.264	35	Bacteria: Bacteroidetes
Phy 654	Transitional	0.010	0.266	37	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 130	Transitional	0.015	0.251	44	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales
Phy 1188	Transitional	0.000	0.243	39	Bacteria: Proteobacteria: Deltaproteobacteria: Bdellovibrionales: Bacteriovoracaceae
Phy 350	Transitional	0.020	0.258	43	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 218	Transitional	0.010	0.251	40	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae
Phy 44	Transitional	0.001	0.254	35	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Ferribacterium
Phy 100	Transitional	0.017	0.264	39	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 337	Transitional	0.003	0.262	33	Bacteria: Bacteroidetes
Phy 504	Transitional	0.002	0.246	37	Bacteria: Spirochaetes: Spirochaetia: Spirochaetales: Leptospiraceae: Leptospira
Phy 437	Transitional	0.003	0.250	36	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae
Phy 96	Transitional	0.003	0.250	36	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Hydrogenophaga
Phy 271	Transitional	0.006	0.248	38	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 272	Transitional	0.011	0.268	34	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Chitinophaga
Phy 112	Transitional	0.002	0.249	36	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 673	Transitional	0.009	0.252	37	Bacteria: Proteobacteria: Betaproteobacteria: Nitrosomonadales: Nitrosomonadaceae
Phy 195	Transitional	0.017	0.264	37	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Nannocystaceae: Nannocystis

Phy 254	Transitional	0.012	0.260	36	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae
Phy 307	Transitional	0.000	0.243	36	Bacteria: Bacteroidetes
Phy 1144	Transitional	0.001	0.241	37	Bacteria
Phy 499	Transitional	0.001	0.243	36	Bacteria: Proteobacteria: Deltaproteobacteria: Bdellovibrionales: Bacteriovoracaceae
Phy 32	Transitional	0.002	0.262	31	Bacteria: Proteobacteria
Phy 717	Transitional	0.010	0.265	33	Bacteria: Proteobacteria
Phy 288	Transitional	0.004	0.243	37	Bacteria: Actinobacteria: Actinobacteria: Acidimicrobidae: Acidimicrobiales: Acidimicrobineae: Iamiaceae: Iamia
Phy 365	Transitional	0.015	0.257	37	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 153	Transitional	0.006	0.243	38	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 441	Transitional	0.004	0.243	37	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae: Runella
Phy 132	Transitional	0.004	0.242	37	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 93	Transitional	0.013	0.248	39	Bacteria: Bacteroidetes
Phy 185	Transitional	0.003	0.243	36	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis
Phy 47	Transitional	0.032	0.254	48	Bacteria: Proteobacteria: Betaproteobacteria
Phy 264	Transitional	0.002	0.237	37	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 23	Continuoulsy abundant	0.007	0.249	35	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 159	Transitional	0.023	0.273	35	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 556	Transitional	0.006	0.253	33	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 213	Transitional	0.007	0.244	36	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sterolibacterium
Phy 171	Transitional	0.020	0.268	34	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 916	Transitional	0.001	0.260	28	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 796	Transitional	0.001	0.254	29	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 21	Continuoulsy abundant	0.015	0.258	33	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 618	Transitional	0.002	0.248	30	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 250	Transitional	0.002	0.254	28	Bacteria: Bacteroidetes
Phy 377	Transitional	0.009	0.254	30	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 832	Transitional	0.004	0.258	27	Bacteria: Planctomycetes: Planctomycetia
Phy 1326	Transitional	0.001	0.259	26	Bacteria: Microgenomates: Microgenomates_genera_incertae_sedis
Phy 277	Transitional	0.011	0.244	33	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sterolibacterium
Phy 34	Transitional	0.017	0.268	29	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 831	Transitional	0.008	0.260	27	Bacteria
Phy 455	Transitional	0.001	0.251	27	Bacteria: Planctomycetes: Planctomycetia: Planctomycetales: Planctomycetaceae: Schlesneria
Phy 1622	Transitional	0.001	0.246	28	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sulfuritalea
Phy 86	Transitional	0.006	0.231	34	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 639	Transitional	0.002	0.230	33	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Pseudomonadaceae: Cellvibrio
Phy 815	Transitional	0.001	0.259	25	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 73	Transitional	0.001	0.237	30	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 135	Transitional	0.027	0.274	31	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 1118	Transitional	0.003	0.260	25	Bacteria: Proteobacteria
Phy 479	Transitional	0.018	0.268	28	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Byssovorax

Phy 242	Transitional	0.023	0.264	31	Bacteria: Proteobacteria: Alphaproteobacteria: Rickettsiales: Rickettsiaceae
Phy 393	Transitional	0.007	0.235	32	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 268	Transitional	0.002	0.240	29	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 123	Transitional	0.026	0.267	31	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Georgfuchsia
Phy 704	Transitional	0.001	0.259	24	Bacteria: Proteobacteria
Phy 186	Transitional	0.009	0.244	30	Bacteria
Phy 708	Transitional	0.001	0.230	31	Bacteria
Phy 223	Transitional	0.004	0.230	32	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 228	Transitional	0.018	0.262	28	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 493	Transitional	0.000	0.231	30	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 500	Transitional	0.001	0.242	27	Bacteria
Phy 230	Transitional	0.039	0.278	35	Bacteria: Chloroflexi
Phy 464	Transitional	0.039	0.262	39	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 431	Transitional	0.015	0.266	26	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 391	Transitional	0.005	0.260	24	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae
Phy 110	Transitional	0.003	0.257	24	Bacteria: Bacteroidetes
Phy 52	Transitional	0.008	0.242	29	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 314	Transitional	0.001	0.237	28	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae
Phy 1325	Transitional	0.043	0.268	39	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 579	Transitional	0.001	0.247	25	Bacteria
Phy 212	Transitional	0.007	0.264	23	Bacteria: Gemmatimonadetes: Gemmatimonadetes: Gemmatimonadales: Gemmatimonadaceae: Gemmatimonas
Phy 462	Transitional	0.011	0.265	24	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae: Solitalea
Phy 438	Transitional	0.001	0.238	27	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae: Pedobacter
Phy 515	Transitional	0.001	0.245	25	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 593	Transitional	0.012	0.258	25	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 161	Transitional	0.000	0.238	26	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 418	Transitional	0.033	0.269	31	Bacteria: Proteobacteria
Phy 758	Transitional	0.024	0.272	26	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 1117	Transitional	0.001	0.255	22	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Soonwooa
Phy 838	Transitional	0.013	0.238	29	Bacteria
Phy 612	Transitional	0.001	0.228	28	Bacteria
Phy 457	Transitional	0.042	0.281	32	Bacteria
Phy 366	Transitional	0.065	0.283	59	Bacteria
Phy 269	Transitional	0.002	0.238	25	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sulfuritalea
Phy 600	Transitional	0.002	0.249	22	Bacteria: Bacteroidetes
Phy 165	Transitional	0.002	0.260	20	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 707	Transitional	0.006	0.258	21	Bacteria: candidate division WPS-1: WPS-1_genera_incertae_sedis
Phy 778	Transitional	0.000	0.240	23	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 358	Transitional	0.002	0.265	19	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 319	Transitional	0.000	0.240	23	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Rubrivivax

Phy 136	Transitional	0.003	0.238	24	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 414	Transitional	0.001	0.228	26	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Ferruginibacter
Phy 224	Transitional	0.002	0.243	22	Bacteria: Proteobacteria: Betaproteobacteria
Phy 154	Transitional	0.010	0.266	20	Bacteria
Phy 666	Transitional	0.000	0.228	25	Bacteria
Phy 793	Transitional	0.009	0.257	21	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 274	Transitional	0.046	0.265	35	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 798	Transitional	0.001	0.245	21	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae
Phy 114	Transitional	0.001	0.240	22	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Thauera
Phy 221	Transitional	0.003	0.237	23	Bacteria: Planctomycetes: Planctomycetia: Planctomycetales: Planctomycetaceae
Phy 20	Continuoulsy abundant	0.001	0.239	22	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sulfuritalea
Phy 282	Transitional	0.004	0.233	24	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sterolibacterium
Phy 449	Transitional	0.005	0.258	19	Bacteria: Bacteroidetes
Phy 62	Transitional	0.017	0.244	25	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 762	Transitional	0.000	0.220	26	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 895	Transitional	0.001	0.232	23	Bacteria: Verrucomicrobia: Opitutae: Puniceicoccales: Puniceicoccaceae
Phy 1428	Transitional	0.004	0.231	24	Bacteria: Proteobacteria: Deltaproteobacteria: Bdellovibrionales: Bdellovibrionaceae: Bdellovibrio
Phy 248	Transitional	0.002	0.257	18	Bacteria: Acidobacteria: Acidobacteria_Gp4: Gp4
Phy 343	Transitional	0.000	0.228	23	Bacteria: Firmicutes: Bacilli: Lactobacillales: Lactobacillaceae: Lactobacillus
Phy 239	Transitional	0.001	0.237	21	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Prevotellaceae: Prevotella
Phy 483	Transitional	0.015	0.268	19	Bacteria: Latescibacteria: Latescibacteria_genera_incertae_sedis
Phy 394	Transitional	0.002	0.232	22	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 557	Transitional	0.015	0.229	26	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sterolibacterium
Phy 175	Transitional	0.023	0.258	22	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 300	Transitional	0.004	0.253	18	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 401	Transitional	0.001	0.234	21	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 795	Transitional	0.005	0.254	18	Bacteria
Phy 244	Transitional	0.031	0.272	22	Bacteria: Bacteroidetes
Phy 58	Continuoulsy abundant	0.042	0.245	33	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 407	Transitional	0.001	0.240	19	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 109	Continuoulsy abundant	0.037	0.271	24	Bacteria: Proteobacteria: Gammaproteobacteria: Oceanospirillales
Phy 339	Transitional	0.045	0.255	32	Bacteria
Phy 348	Transitional	0.003	0.254	17	Bacteria
Phy 739	Transitional	0.004	0.242	19	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Wandonia
Phy 56	Transitional	0.002	0.214	25	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Chryseobacterium
Phy 635	Transitional	0.004	0.230	21	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Curvibacter
Phy 151	Transitional	0.004	0.251	17	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Ideonella
Phy 290	Transitional	0.002	0.255	16	Bacteria: Bacteroidetes
Phy 179	Transitional	0.003	0.254	16	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Sediminibacterium
Phy 83	Transitional	0.003	0.246	17	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Terrimonas

Phy 33	Transitional	0.014	0.236	21	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 406	Transitional	0.012	0.265	16	Bacteria: Bacteroidetes
Phy 267	Transitional	0.026	0.247	22	Bacteria: Proteobacteria
Phy 1361	Transitional	0.000	0.224	20	Bacteria
Phy 270	Transitional	0.012	0.263	16	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 447	Transitional	0.002	0.249	16	Bacteria
Phy 173	Transitional	0.025	0.240	23	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae: Rudaea
Phy 222	Transitional	0.006	0.240	17	Bacteria: Verrucomicrobia: Subdivision3: Subdivision3_genera_incertae_sedis
Phy 327	Transitional	0.012	0.254	16	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae
Phy 330	Transitional	0.004	0.251	15	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Pelomonas
Phy 480	Transitional	0.003	0.250	15	Bacteria
Phy 15	Continuoulsy abundant	0.011	0.239	18	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Simplicispira
Phy 1107	Transitional	0.001	0.227	18	Unclassified
Phy 352	Transitional	0.005	0.244	16	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 231	Transitional	0.001	0.231	17	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Zoogloea
Phy 320	Transitional	0.000	0.235	16	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 99	Transitional	0.003	0.238	16	Bacteria: Firmicutes: Clostridia: Clostridiales: Natranaerovirga
Phy 298	Transitional	0.002	0.237	16	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 629	Transitional	0.014	0.269	14	Bacteria: Planctomycetes: Planctomycetia: Planctomycetales: Planctomycetaceae
Phy 115	Transitional	0.004	0.251	14	Bacteria: Proteobacteria
Phy 475	Transitional	0.008	0.234	17	Bacteria
Phy 460	Transitional	0.000	0.235	15	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Oxalobacteraceae
Phy 745	Transitional	0.001	0.229	16	Bacteria: Bacteroidetes
Phy 417	Transitional	0.014	0.238	17	Bacteria: Proteobacteria: Alphaproteobacteria: Rickettsiales: Rickettsiaceae: Rickettsia
Phy 283	Transitional	0.015	0.273	13	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Ferruginibacter
Phy 767	Transitional	0.011	0.246	15	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Cryomorpha
Phy 523	Transitional	0.026	0.260	16	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 792	Transitional	0.002	0.234	15	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 573	Transitional	0.005	0.230	16	Bacteria
Phy 284	Transitional	0.003	0.233	15	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Haliangiaceae: Haliangium
Phy 68	Transitional	0.000	0.230	15	Bacteria: Proteobacteria: Gammaproteobacteria: Methylococcales: Methylococcaceae: Methylococcus
Phy 763	Transitional	0.000	0.223	16	Bacteria
Phy 202	Transitional	0.000	0.212	18	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 306	Transitional	0.010	0.238	15	Bacteria: Proteobacteria: Deltaproteobacteria: Bdellovibrionales: Bacteriovoracaceae: Peredibacter
Phy 375	Transitional	0.011	0.238	15	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Cystobacterineae: Cystobacteraceae
Phy 411	Transitional	0.004	0.244	13	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 1017	Transitional	0.007	0.239	14	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 605	Transitional	0.006	0.235	14	Bacteria
Phy 341	Transitional	0.029	0.240	18	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 54	Transitional	0.011	0.240	14	Bacteria: Proteobacteria

Phy 642	Transitional	0.005	0.233	14	Unclassified
Phy 547	Transitional	0.006	0.250	12	Bacteria: Proteobacteria
Phy 529	Transitional	0.017	0.265	12	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 415	Transitional	0.004	0.245	12	Bacteria: Proteobacteria: Alphaproteobacteria: Rickettsiales: Rickettsiaceae
Phy 510	Transitional	0.002	0.234	13	Unclassified
Phy 31	Transitional	0.028	0.253	15	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Rhodoferax
Phy 9	Transitional	0.000	0.218	15	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 177	Transitional	0.001	0.218	15	Bacteria
Phy 1359	Transitional	0.000	0.228	13	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Ferruginibacter
Phy 1097	Transitional	0.000	0.227	13	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 188	Transitional	0.000	0.234	12	Bacteria: Chloroflexi
Phy 39	Continuoulsy abundant	0.009	0.235	13	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Variovorax
Phy 360	Transitional	0.010	0.217	16	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 95	Transitional	0.006	0.240	12	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 597	Transitional	0.002	0.235	12	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 351	Transitional	0.006	0.239	12	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 646	Transitional	0.000	0.217	14	Bacteria
Phy 143	Transitional	0.003	0.232	12	Bacteria: Proteobacteria: Gammaproteobacteria: Candidatus Carsonella
Phy 966	Transitional	0.000	0.221	13	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae: Cytophaga
Phy 522	Transitional	0.002	0.231	12	Bacteria: Bacteroidetes
Phy 301	Transitional	0.002	0.230	12	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 18	Transitional	0.002	0.236	11	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Paludibacter
Phy 41	Transitional	0.001	0.243	10	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Uliginosibacterium
Phy 11	Transitional	0.009	0.217	14	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 1248	Transitional	0.002	0.242	10	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 701	Transitional	0.000	0.221	12	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 168	Transitional	0.022	0.258	11	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales
Phy 26	Transitional	0.017	0.224	14	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 37	Continuoulsy abundant	0.011	0.231	12	Bacteria: Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae
Phy 688	Transitional	0.050	0.271	16	Bacteria: Acidobacteria: Acidobacteria_Gp4: Gp4
Phy 1101	Transitional	0.009	0.236	11	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 458	Transitional	0.002	0.237	10	Bacteria: Actinobacteria: Actinobacteria: Coriobacteridae: Coriobacteriales: Coriobacterineae: Coriobacteriaceae: Collinsella
Phy 380	Transitional	0.001	0.248	9	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Jahnella
Phy 568	Transitional	0.001	0.247	9	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Sorangium
Phy 786	Transitional	0.016	0.242	11	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 631	Transitional	0.013	0.264	9	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae: Haliscomenobacter
Phy 676	Transitional	0.000	0.216	12	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 40	Continuoulsy abundant	0.018	0.254	10	Bacteria: Spirochaetes: Spirochaetia: Spirochaetales: Leptospiraceae: Turneriella
Phy 400	Transitional	0.017	0.252	10	Bacteria: Proteobacteria: Gammaproteobacteria: Thiotrichales: Thiotrichales_incertae_sedis: Caedibacter
Phy 81	Transitional	0.001	0.231	10	Bacteria: Acidobacteria: Holophagae: Holophagales: Holophagaceae: Geothrix

Phy 566	Transitional	0.000	0.241	9	Bacteria: Bacteroidetes: Bacteroidetes_incertae_sedis: Ohtaekwangia
Phy 1185	Transitional	0.000	0.240	9	Bacteria: Proteobacteria
Phy 550	Transitional	0.000	0.220	11	Bacteria: Proteobacteria: Alphaproteobacteria: Caulobacterales: Hyphomonadaceae
Phy 29	Transitional	0.003	0.215	12	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 877	Transitional	0.001	0.229	10	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 148	Transitional	0.023	0.246	11	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Rivibacter
Phy 88	Transitional	0.002	0.220	11	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae
Phy 180	Transitional	0.000	0.234	9	Bacteria: Proteobacteria: Alphaproteobacteria: Rhodobacterales: Rhodobacteraceae: Pseudorhodobacter
Phy 712	Transitional	0.000	0.224	10	Bacteria: Proteobacteria
Phy 487	Transitional	0.008	0.241	9	Bacteria
Phy 383	Transitional	0.001	0.221	10	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 142	Transitional	0.000	0.243	8	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 643	Transitional	0.000	0.219	10	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Sorangiineae: Polyangiaceae: Jahnella
Phy 17	Transitional	0.000	0.219	10	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Perlucidibaca
Phy 685	Transitional	0.002	0.221	10	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 722	Transitional	0.001	0.229	9	Bacteria: Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae: Clostridium XI
Phy 532	Transitional	0.007	0.246	8	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 1328	Transitional	0.000	0.225	9	Bacteria
Phy 108	Transitional	0.000	0.224	9	Bacteria: Proteobacteria: Deltaproteobacteria: Desulfuromonadales: Geobacteraceae: Geobacter
Phy 764	Transitional	0.007	0.227	9	Bacteria: Proteobacteria
Phy 413	Transitional	0.024	0.263	8	Bacteria: Ignavibacteriae: Ignavibacteria: Ignavibacteriales: Ignavibacteriaceae: Ignavibacterium
Phy 993	Transitional	0.001	0.229	8	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae
Phy 503	Transitional	0.000	0.217	9	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Oxalobacteraceae
Phy 291	Transitional	0.000	0.227	8	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Terrimonas
Phy 80	Transitional	0.000	0.216	9	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 961	Transitional	0.028	0.250	9	Bacteria
Phy 1353	Transitional	0.000	0.207	10	Bacteria
Phy 614	Transitional	0.001	0.226	8	Bacteria
Phy 398	Transitional	0.000	0.238	7	Bacteria: Nitrospirae: Nitrospira: Nitrospirales: Nitrospiraceae: Nitrospira
Phy 1	Continuoulsy abundant	0.004	0.227	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Rhodoferax
Phy 844	Transitional	0.004	0.224	8	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 684	Transitional	0.000	0.234	7	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 587	Transitional	0.000	0.232	7	Bacteria: Proteobacteria: Betaproteobacteria: Neisseriales: Neisseriaceae: Aquitalea
Phy 36	Continuoulsy abundant	0.001	0.219	8	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 777	Transitional	0.000	0.230	7	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 217	Transitional	0.003	0.252	6	Bacteria: Proteobacteria: Gammaproteobacteria: Chromatiales: Ectothiorhodospiraceae
Phy 194	Transitional	0.001	0.217	8	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae
Phy 214	Transitional	0.006	0.221	8	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 117	Transitional	0.045	0.243	11	Bacteria
Phy 636	Transitional	0.000	0.213	8	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Limnohabitans

Phy 16	Transitional	0.003	0.214	8	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 98	Transitional	0.002	0.214	8	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Haliangiaceae: Haliangium
Phy 294	Transitional	0.000	0.238	6	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis
Phy 521	Transitional	0.000	0.238	6	Bacteria: Acidobacteria
Phy 361	Transitional	0.006	0.226	7	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Aquabacterium
Phy 553	Transitional	0.006	0.225	7	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 919	Transitional	0.002	0.221	7	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 322	Transitional	0.016	0.231	7	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 10	Continuoulsy abundant	0.006	0.219	7	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 215	Transitional	0.003	0.231	6	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 211	Transitional	0.005	0.232	6	Bacteria: Proteobacteria: Betaproteobacteria: Methylophilales: Methylophilaceae: Methylotenera
Phy 12	Continuoulsy abundant	0.000	0.210	7	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis: Aquabacterium
Phy 986	Transitional	0.000	0.210	7	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 187	Transitional	0.000	0.242	5	Bacteria: Bacteroidetes
Phy 234	Transitional	0.002	0.222	6	Bacteria: Bacteroidetes
Phy 1366	Transitional	0.002	0.221	6	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 379	Transitional	0.000	0.218	6	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 137	Transitional	0.000	0.218	6	Bacteria: Proteobacteria
Phy 1133	Transitional	0.008	0.211	7	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 60	Continuoulsy abundant	0.005	0.218	6	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 6	Continuoulsy abundant	0.072	0.250	18	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae
Phy 164	Transitional	0.034	0.238	7	Bacteria
Phy 51	Transitional	0.000	0.231	5	Bacteria: Fusobacteria: Fusobacteriia: Fusobacteriales
Phy 344	Transitional	0.000	0.230	5	Bacteria: Proteobacteria
Phy 652	Transitional	0.003	0.214	6	Bacteria
Phy 632	Transitional	0.003	0.212	6	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 35	Transitional	0.012	0.219	6	Bacteria: Bacteroidetes
Phy 149	Transitional	0.000	0.225	5	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 207	Transitional	0.001	0.197	7	Bacteria: Proteobacteria
Phy 450	Transitional	0.000	0.225	5	Unclassified
Phy 57	Continuoulsy abundant	0.011	0.231	5	Bacteria: Proteobacteria: Alphaproteobacteria: Rhodospirillales: Acetobacteraceae: Stella
Phy 170	Transitional	0.011	0.231	5	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Filimonas
Phy 182	Transitional	0.001	0.220	5	Bacteria: Proteobacteria
Phy 312	Transitional	0.053	0.247	8	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Cryomorpha
Phy 225	Transitional	0.008	0.253	4	Bacteria: Verrucomicrobia: Subdivision3: Subdivision3_genera_incertae_sedis
Phy 1730	Transitional	0.000	0.214	5	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Simplicispira
Phy 1481	Transitional	0.000	0.199	6	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 61	Continuoulsy abundant	0.001	0.238	4	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Terrimonas
Phy 43	Transitional	0.011	0.222	5	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae: Leadbetterella
Phy 491	Transitional	0.000	0.210	5	Bacteria: Firmicutes: Bacilli: Lactobacillales: Lactobacillaceae: Lactobacillus

Phy 1623	Transitional	0.002	0.212	5	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 121	Transitional	0.002	0.212	5	Bacteria: Proteobacteria
Phy 129	Transitional	0.008	0.242	4	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae: Haliscomenobacter
Phy 48	Transitional	0.003	0.212	5	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 259	Transitional	0.000	0.210	5	Bacteria: Proteobacteria: Gammaproteobacteria: Candidatus Carsonella
Phy 5	Continuoulsy abundant	0.021	0.229	5	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales
Phy 253	Transitional	0.004	0.211	5	Bacteria: Bacteroidetes
Phy 69	Transitional	0.001	0.195	6	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: PorPhy romonadaceae: Parabacteroides
Phy 184	Transitional	0.072	0.272	11	Bacteria: Bacteroidetes
Phy 174	Transitional	0.000	0.231	4	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae: Ferruginibacter
Phy 243	Transitional	0.000	0.226	4	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 1047	Transitional	0.004	0.207	5	Bacteria: Proteobacteria
Phy 45	Continuoulsy abundant	0.000	0.225	4	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Sulfuritalea
Phy 251	Transitional	0.003	0.203	5	Bacteria: Proteobacteria
Phy 25	Transitional	0.002	0.202	5	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Dechloromonas
Phy 753	Transitional	0.001	0.199	5	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 296	Transitional	0.000	0.196	5	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Haliangiaceae: Haliangium
Phy 317	Transitional	0.004	0.219	4	Bacteria: Firmicutes: Negativicutes: Selenomonadales: Veillonellaceae: Veillonella
Phy 46	Transitional	0.000	0.196	5	Bacteria: Proteobacteria: Gammaproteobacteria
Phy 204	Transitional	0.000	0.194	5	Bacteria: Proteobacteria
Phy 373	Transitional	0.001	0.212	4	Bacteria: Verrucomicrobia: Subdivision3: Subdivision3_genera_incertae_sedis
Phy 1080	Transitional	0.000	0.210	4	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 985	Transitional	0.000	0.210	4	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 85	Continuoulsy abundant	0.000	0.245	3	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 1510	Transitional	0.001	0.208	4	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Nannocystaceae: Nannocystis
Phy 511	Transitional	0.001	0.208	4	Bacteria
Phy 107	Transitional	0.000	0.207	4	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Limnohabitans
Phy 833	Transitional	0.000	0.239	3	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 90	Transitional	0.000	0.201	4	Bacteria: Bacteroidetes
Phy 183	Transitional	0.009	0.242	3	Bacteria
Phy 4	Continuoulsy abundant	0.003	0.201	4	Bacteria: Proteobacteria: Epsilonproteobacteria: Campylobacterales: Campylobacteraceae: Arcobacter
Phy 172	Transitional	0.000	0.226	3	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Variovorax
Phy 1232	Transitional	0.001	0.197	4	Bacteria: SR1: SR1_genera_incertae_sedis
Phy 266	Transitional	0.007	0.196	4	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Oxalobacteraceae
Phy 104	Transitional	0.000	0.217	3	Bacteria: Proteobacteria: Gammaproteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
Phy 28	Continuoulsy abundant	0.000	0.191	4	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Aeromonas
Phy 444	Transitional	0.005	0.194	4	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 1135	Transitional	0.000	0.216	3	Bacteria
Phy 486	Transitional	0.007	0.220	3	Bacteria: Actinobacteria: Actinobacteria: Actinobacteridae: Bifidobacteriales: Bifidobacteriaceae: Bifidobacterium
Phy 388	Transitional	0.004	0.216	3	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis

Phy 297	Transitional	0.000	0.212	3	Bacteria: Proteobacteria: Betaproteobacteria: Nitrosomonadales: Nitrosomonadaceae: Nitrosomonas
Phy 443	Transitional	0.000	0.211	3	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 77	Transitional	0.001	0.210	3	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 141	Transitional	0.000	0.209	3	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Sphingobacteriaceae
Phy 426	Transitional	0.000	0.208	3	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 1449	Transitional	0.000	0.207	3	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 507	Transitional	0.001	0.207	3	Bacteria: Actinobacteria: Actinobacteria: Coriobacteridae: Coriobacteriales: Coriobacterineae: Coriobacteriaceae: Olsenella
Phy 1250	Transitional	0.006	0.209	3	Bacteria: Actinobacteria: Actinobacteria: Acidimicrobidae: Acidimicrobiales: Acidimicrobineae: Acidimicrobiaceae: Ilumatobacter
Phy 442	Transitional	0.000	0.203	3	Bacteria: Proteobacteria: Epsilonproteobacteria
Phy 891	Transitional	0.000	0.200	3	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Chitinophagaceae
Phy 75	Transitional	0.005	0.201	3	Bacteria: Candidatus Saccharibacteria: Saccharibacteria_genera_incertae_sedis
Phy 55	Transitional	0.005	0.200	3	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 87	Transitional	0.000	0.227	2	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 422	Transitional	0.052	0.241	3	Bacteria
Phy 169	Transitional	0.000	0.224	2	Bacteria: Proteobacteria: Betaproteobacteria
Phy 429	Transitional	0.000	0.220	2	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae
Phy 97	Continuoulsy abundant	0.000	0.215	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis
Phy 3	Continuoulsy abundant	0.002	0.211	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 387	Transitional	0.000	0.208	2	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae
Phy 105	Transitional	0.000	0.208	2	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 247	Transitional	0.000	0.205	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Burkholderiales_incertae_sedis
Phy 66	Transitional	0.003	0.207	2	Bacteria: Bacteroidetes
Phy 162	Transitional	0.000	0.202	2	Bacteria: Fusobacteria: Fusobacteriia: Fusobacteriales: Leptotrichiaceae
Phy 59	Continuoulsy abundant	0.000	0.202	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Acidovorax
Phy 84	Transitional	0.000	0.200	2	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Propionivibrio
Phy 200	Transitional	0.000	0.198	2	Bacteria: Proteobacteria: Betaproteobacteria
Phy 640	Transitional	0.000	0.196	2	Bacteria: Proteobacteria: Alphaproteobacteria: Sphingomonadales: Sphingomonadaceae: Novosphingobium
Phy 219	Transitional	0.004	0.198	2	Bacteria: Bacteroidetes
Phy 78	Transitional	0.001	0.196	2	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Limnohabitans
Phy 144	Transitional	0.000	0.195	2	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 65	Transitional	0.000	0.191	2	Bacteria: Proteobacteria
Phy 396	Transitional	0.008	0.193	2	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales: Saprospiraceae
Phy 116	Transitional	0.000	0.188	2	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 371	Transitional	0.000	0.188	2	Bacteria: Bacteroidetes
Phy 408	Transitional	0.001	0.183	2	Bacteria: Proteobacteria: Gammaproteobacteria: Oceanospirillales
Phy 113	Transitional	0.000	0.179	2	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 477	Transitional	0.000	0.179	2	Bacteria: Proteobacteria
Phy 106	Transitional	0.000	0.178	2	Bacteria: Proteobacteria: Gammaproteobacteria: Aeromonadales: Aeromonadaceae: Tolumonas
Phy 71	Transitional	0.000	0.176	2	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Azonexus
Phy 72	Continuoulsy abundant	0.000	0.175	2	Bacteria: Bacteroidetes: Cytophagia: Cytophagales

Phy 586	Transitional	0.004	0.174	2	Bacteria: Firmicutes: Bacilli: Bacillales: StaPhy lococcaceae: StaPhy lococcus
Phy 131	Transitional	0.000	0.169	2	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales: Bacteroidaceae: Bacteroides
Phy 209	Transitional	0.004	0.162	2	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 419	Transitional	0.090	0.260	27	Bacteria: Verrucomicrobia: Opitutae: Opitutales: Opitutaceae: Opitutus
Phy 544	Transitional	0.000	0.213	1	Bacteria: Acidobacteria: Acidobacteria_Gp4: Gp4
Phy 423	Transitional	0.000	0.211	1	Bacteria: Proteobacteria: Betaproteobacteria
Phy 535	Transitional	0.000	0.210	1	Bacteria: Parcubacteria: Parcubacteria_genera_incertae_sedis
Phy 323	Transitional	0.000	0.208	1	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Verrucomicrobium
Phy 476	Transitional	0.000	0.205	1	Bacteria: Bacteroidetes
Phy 421	Transitional	0.000	0.203	1	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 260	Transitional	0.000	0.202	1	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 376	Transitional	0.000	0.197	1	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 420	Transitional	0.000	0.196	1	Bacteria: Bacteroidetes: Sphingobacteriia: Sphingobacteriales
Phy 336	Transitional	0.000	0.196	1	Bacteria: Bacteroidetes: Cytophagia: Cytophagales
Phy 855	Transitional	0.000	0.195	1	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 308	Transitional	0.000	0.194	1	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 236	Transitional	0.000	0.193	1	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Simplicispira
Phy 292	Transitional	0.000	0.193	1	Bacteria
Phy 280	Transitional	0.000	0.189	1	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 946	Transitional	0.000	0.188	1	Bacteria: Proteobacteria: Alphaproteobacteria: Rhodobacterales: Rhodobacteraceae: Rhodobacter
Phy 662	Transitional	0.000	0.183	1	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Comamonadaceae: Hydrogenophaga
Phy 382	Transitional	0.000	0.181	1	Bacteria: Proteobacteria: Epsilonproteobacteria
Phy 27	Transitional	0.000	0.180	1	Bacteria: Bacteroidetes: Bacteroidia: Bacteroidales
Phy 49	Transitional	0.000	0.180	1	Bacteria: Proteobacteria: Betaproteobacteria: Rhodocyclales: Rhodocyclaceae: Dechloromonas
Phy 79	Transitional	0.000	0.180	1	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Cryomorphaceae: Cryomorpha
Phy 374	Transitional	0.000	0.179	1	Bacteria: Proteobacteria: Deltaproteobacteria
Phy 138	Transitional	0.000	0.179	1	Bacteria: Verrucomicrobia: Verrucomicrobiae: Verrucomicrobiales: Verrucomicrobiaceae: Prosthecobacter
Phy 24	Transitional	0.000	0.178	1	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 42	Continuoulsy abundant	0.000	0.178	1	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Flammeovirgaceae: Fabibacter
Phy 278	Transitional	0.000	0.175	1	Bacteria: Bacteroidetes
Phy 569	Transitional	0.000	0.171	1	Bacteria
Phy 570	Transitional	0.000	0.168	1	Bacteria: Bacteroidetes: Flavobacteriia: Flavobacteriales: Flavobacteriaceae: Flavobacterium
Phy 178	Transitional	0.000	0.167	1	Bacteria: Bacteroidetes: Cytophagia: Cytophagales: Cytophagaceae
Phy 784	Transitional	0.000	0.165	1	Bacteria: Proteobacteria
Phy 13	Continuoulsy abundant	0.000	0.164	1	Bacteria: Proteobacteria: Deltaproteobacteria: Myxococcales: Nannocystineae: Nannocystaceae: Nannocystis
Phy 608	Transitional	0.000	0.162	1	Bacteria: Proteobacteria
Phy 190	Transitional	0.000	0.148	1	Bacteria: Bacteroidetes
Phy 773	Transitional	0.000	0.139	1	Bacteria: Proteobacteria

ANNEX II:

SUPPLEMENTARY INFORMATION FOR CHAPTER 3

Table A.II.1

Characteristics of the two different anaerobic inoculum types for the BMP experiments. Standard errors are shown behind values (n=3).

	CSTR sludge			UASB sludge			Unit
pH	6.85	±	0.01	8.31	±	0.01	-
Volatile fatty acids	0	±	0	0	±	0	mg COD L^{-1}
Sulfate	0	±	0	0	±	0	mg L^{-1}
Phosphate	n.a.			365	±	14	mg L^{-1}
Conductivity	4.8	±	0.0	35.3	±	0.2	mS cm^{-1}
Total solids	51.5	±	1.0	80.9	±	0.3	g TS kg^{-1}
Volatile solids	42.8	±	0.9	39.9	±	0.2	g VS kg^{-1}
Total ammonia nitrogen	100	±	12	4335	±	32	mg NH_4^+-N kg^{-1}

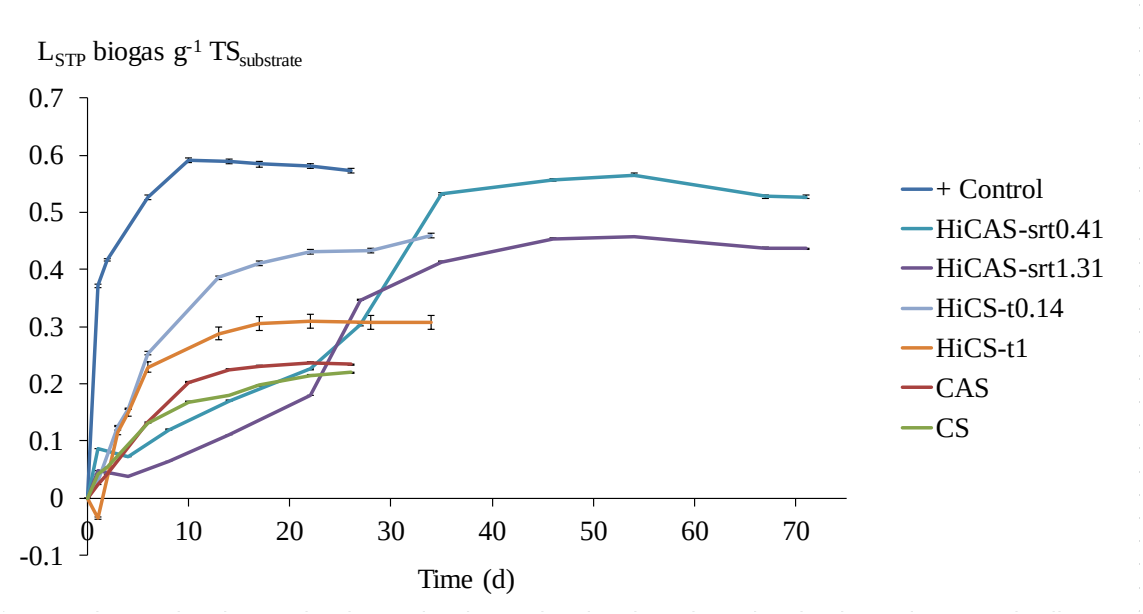


Figure A.II.1

Biogas production of the different sludge types of the SBR reactors, expressed as liters of biogas produced at standard temperature and pressure (STP; 273.15 K and 100 kPa) per gram of substrate TS.

CURRICULUM VITAE

Personal information

Surname: Meerburg
Name: Francis

Address: Steendam 80,
9000 Ghent
Belgium
Phone: +32 (0) 499 60 40 86
E-mail: francis.meerburg@ugent.be

Education

2016-now	Research study responsible Aquafin NV, Belgium
2012-2016	PhD: Doctor in Applied Biological Sciences, Doctoral School of Bioscience Engineering Ghent University, BELGIUM
2011-2012	Environmental Engineering and Science Stanford University, California, USA Degree (2012): M.S. in Environmental Engineering and Science
2009-2011	Master of Biology Majors: Biodiversity and Ecology Ghent University, Belgium Degree (2011): MSc in Biology
2006-2009	Program: Bachelor of Biology Ghent University, Belgium Degree (2009): BSc in Biology
2001-2006	Steiner Secondary School, Ghent, Belgium

Academic research activities

2012-now	Scientific collaborator at Laboratory of Microbial Ecology and Technology PhD topic: '*High-rate activated sludge systems to maximize recovery of energy from wastewater - Microbial ecology and novel operational strategies*' (FWO scholarship)
2011-2012	Independent research project: '*Optimization of a PHB extraction protocol from a mixed culture of type-II methanotrophs*'. Supervisor: Prof. dr. Craig Criddle, Stanford University, California, USA.
2010-2011	Master thesis: '*Biologically produced manganese oxides (Bio-MnOx) and silver nanoparticles (Bio-Ag0) for the catalytic degradation of organic micropollutants*'. Promoters: Prof. dr. ir. Willy Verstraete and prof. dr. ir. Nico Boon, Laboratory for Microbial Ecology and Technology. Prof. dr. Paul De Vos, Laboratory of Microbiology, Ghent University, Belgium.
2008-2009	Bachelor thesis: '*Biostratigraphy of Chitinozoa in the P. linearis zone, Late Ordovicium, in Bornholm (Denmark)*'. Promoter: Prof. dr. Jacques Verniers, Research group Paleontology, Ghent University, Belgium.

Research interests

Research and development, Environmental engineering, Bioscience engineering, Water and wastewater treatment, Resource recovery, Energy recovery, Microbial community analysis

Scientific awards

2016 Prijs Ernest Du Bois – doctoraat (Koning Boudewijnstichting) for the project "Hoogbelast actief slib voor maximalisatie van de herwinning van energie uit afvalwater voor een meer duurzame, energie-autonome en veiligere waterzuivering, en praktische toepassing in ontwikkelings- en ontwikkelde landen." Prize for young engineers (in the last two years of doctoral study) who write a doctoral thesis on the theme of water and its availability on a global level.

2011 Best thesis in Master of Biology (Pierre Verkerk award) 2010-2011, Ghent University, with "Biologically produced manganese oxides (Bio-MnOx) and silver nanoparticles (Bio-Ag0) for the catalytic degradation of organic micropollutants."

2011 Poster award at First International Symposium on Microbial Resource Management in Biotechnology (30 June – 1 July 2011), with "Biogenic manganese oxides and silver nanoparticles for the catalytic degradation of organic micropollutants." First prize for category "Nutrients in Wastewater."

Grants and funding

2016 Prijs Ernest Du Bois – doctoraat (Koning Boudewijnstichting) for the project "*Hoogbelast actief slib voor maximalisatie van de herwinning van energie uit afvalwater voor een meer duurzame, energie-autonome en veiligere waterzuivering, en praktische toepassing in ontwikkelings- en ontwikkelde landen*." Prize for young engineers (in the last two years of doctoral study) who write a doctoral thesis on the theme of water and its availability on a global level. *€ 20 000, used for PhD research.*

2015 Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO-Vlaanderen). "Krediet voor lang verblijf in het buitenland." *€ 4 884, used for a three-month research stay in Washington, D.C.*

2012-2016 Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO-Vlaanderen). "Aspirant." Personal grant for PhD research.

2011-2012 Belgian American Education Foundation (BAEF) – "National Lottery fellow." Grant for the support of exchanging university students, scientists and scholars between the United States and Belgium. *$ 45 000, used for a Master's study in Stanford University, California.*

Publications

A1 – Journals cited in the Journal Citation Reports (Web of Science)

Rahman, A., **Meerburg, F. A.**, Ravadagundhi, S., Wett, B., Jimenez, J., Bott, C., Al-Omari, A., Riffat, R., Murthy, S. & De Clippeleir, H. (2016). Bioflocculation management through high-rate contact-

stabilization: A promising technology to recover organic carbon from low-strength wastewater. *Water Research* 104: 485-496.

Meerburg, F. A., Boon, N., Van Winckel, T., Pauwels, K. T. G. & Vlaeminck, S. E. (2016). Live fast, die young: Optimizing retention times in high-rate contact stabilization for maximal recovery of organics from wastewater. *Environmental Science & Technology* 50(17): 9781-9790.

Meerburg, F. A., Vlaeminck, S. E., Roume, H., Seuntjens, D., Pieper, D. H., Jauregui, R., Vilchez-Vargas, R. & Boon, N. (2016). High-rate activated sludge communities have a distinctly different structure compared to low-rate sludge communities, and are less sensitive towards environmental and operational variables. *Water Research* 100: 137-145.

Meerburg, F. A., Boon, N., Van Winckel, T., Vercamer, J. A. R., Nopens, I. & Vlaeminck, S. E. (2015). Toward energy-neutral wastewater treatment: A high-rate contact stabilization process to maximally recover sewage organics. *Bioresource Technology* 179: 373-381.

Courtens, E. N. P.*, **Meerburg, F. A.***, Mausen, V. & Vlaeminck, S. E. (2014). When the smoke disappears: Dealing with extinguishing chemicals in firefighting wastewater. *Water Science & Technology* 69(8): 1720-1727. (* Equally contributed)

Meerburg, F. A., Hennebel, T., Vanhaecke, L., Verstraete, W. & Boon, N. (2012). Diclofenac and 2-anilinophenylacetate degradation by combined activity of biogenic manganese oxides and silver. *Microbial Biotechnology* 5(3), p.388-395.

A2 – International, peer-reviewed journals, not part of (A1)

Nansubuga, I., **Meerburg, F. A.**, Banadda, N., Rabaey, K. & Verstraete, W. (2015). A two-stage decentralised system combining high rate activated sludge (HRAS) with alternating charcoal filters (ACF) for treating small community sewage to reusable standards for agriculture. *African Journal of Biotechnology* 14(7): 593-603.

A4 – National, non peer-reviewed journals

(see Public outreach)

C1 – Full articles in proceedings of scientific conferences

Meerburg, F. A., Rahman, A., Van Winckel, T., Pauwels, K., De Clippeleir, H., Al-Omari, A., Murthy, S., Boon, N. & Vlaeminck, S. E. (2016). "Fast and furious": Optimization and validation of high-rate contact stabilization (HiCS) for recovery of organics from sewage. In *WEF/IWA Nutrient Removal and Recovery 2016 Conference*, Denver, Colorado (U.S.A.), 10 - 13 July 2016.

Seuntjens, D., **Meerburg, F. A.**, Vlaeminck, S. E., Roume, H., Pieper, D., Jauregui, R., Vilchez-Vargas, R. & Boon, N. (2016). Microbial ecology of high-rate versus conventional activated sludge: Environmental and operational parameters shape microbial structure, co-occurrence and functionality. In *WEF/IWA Nutrient Removal and Recovery 2016 Conference*, Denver, Colorado (U.S.A.), 10 - 13 July 2016.

Rahman, A., **Meerburg, F. A.**, Ravadagundhi, S., Jimenez, J. A., Wett, B., Al-Omari, A. A., Bott, C., Riffat, R., Murthy, S. & De Clippeleir, H. (2016). Management of bioflocculation through high-rate contact-stabilization: A promising technology to recover carbon from low-strength wastewater. In *WEF/IWA Nutrient Removal and Recovery 2016 Conference*, Denver, Colorado (U.S.A.), 10 - 13 July 2016.

Meerburg, F. A., Boon, N., Van Winckel, T., Pauwels, K. & Vlaeminck, S. E. V. (2015). The age of 'wastewater mining': Selection for sludge with a maximum capture potential for organics in a high-rate contact stabilization system. *1st IWA Resource Recovery Conference*, Ghent, Belgium, 30 August - 2 September 2015.

Meerburg, F. A., Vlaeminck, S. E., Vercamer, J. A. R. & Boon, N. (2014). Turn it up! High-load Contact Stabilization (HiCS) is a valuable activated sludge process for maximizing sludge production from sewage. *In 2nd IWA Specialist Conference on EcoTechnologies for Sewage Treatment Plants 2014 "EcoSTP2014"*, Verona, Italy, 23 – 25 June 2014.

Vlaeminck, S. E., **Meerburg, F. A.**, Seuntjens, D., Pintucci, C., Rabaey, K. & Boon, N. (2013). Green fertilizer upcycling from manure through ManureEcoMine: Technological, economic and environmental sustainability demonstration. *ManuREsource: International conference on manure management and valorization*, Bruges, Belgium, 5 – 6 December 2013.

Courtens, E. N. P.*, **Meerburg, F. A.***, Mausen, V. & Vlaeminck, S. E. (2013). When the smoke disappears: Dealing with extinguishing chemicals in firefighting wastewater. *3rd IWA Benelux Young Water Professionals Regional Conference*, Belval, Luxembourg, 2 – 4 October 2013. (* Equally contributed)

Conferences and symposia

Active participation

(see C1 Publications)

Poster contributions

Vlaeminck, S. E., **Meerburg, F. A.**, Vercamer, J. A. R. & Boon, N. (2014). How to recover organics from sewage: High-load contact stabilization as a valuable alternative to the A-stage activated sludge process. *Activated Sludge - 100 Years and Counting*, Essen, Germany, 12 - 14 June 2014.

Meerburg, F. A., Vlaeminck, S. E., Vercamer, J., Verliefde, A. & Boon, N. (2014). High-load contact stabilization is a valuable alternative to high-load activated sludge for pre-treatment of sewage. *AOG RENEW meeting 21 January 2014*, Ghent University, Belgium.

Meerburg, F. A., Vlaeminck, S. E., Vercamer, J. A. R. & Boon, N. (2013). High-rate contact stabilization is a feasible alternative to high-rate activated sludge for scavenging sewage organics. *3rd IWA Benelux Young Water Professionals Regional Conference*, Belval, Luxembourg, 2 - 4 October 2013.

Meerburg, F. A., Hennebel, T., Verstraete, W., Boon, N. (2011). Biogenic manganese oxides and silver nanoparticles for the catalytic degradation of micropollutants. *First International Symposium on Microbial Resource Management in Biotechnology: Concepts & Applications.* Ghent, Belgium, June 30th – July 1st.

Public outreach

Muys, M., **Meerburg, F. A.** & Vlaeminck, S. E. (2015). Gaan microbiële eiwitten de wereld redden? *MO* Mondiaal Nieuws* (Available at http://mo.be/analyse/gaan-microbi-le-eiwitten-de-wereld-redden).

Muys, M., **Meerburg, F. A.** & Vlaeminck, S. E. (2015). Microbiële eiwitbronnen: Smullen van bacteriën. *EOS magazine* 32(7/8): 92-94.

Van Nevel, S. & **Meerburg, F. A.** (2014). Kwaliteitscontrole - Microben uit de kraan. *EOS magazine* 31(9): 50-51.

Meerburg, F. A. and Haelewaters, D. (2013). Glaasje rioolwater? - Afvalwater als bron voor energie, meststoffen en drinkwater. *EOS Magazine* 30(1), pp. 84-88.

Haelewaters, D., **Meerburg, F. A.** (2012). Afvalwaterzuivering door de eeuwen heen. *SciLogs blogs* (Available at http://www.scilogs.be/l-i-f-e/afvalwaterzuivering-door-de-eeuwen-heen/).

Various blog posts about scientific questions on https://www.ikhebeenvraag.be. Example and post on Twitter: https://twitter.com/ihevraag/status/661490753139564544

Collaboration in international research projects

2015 Foreign research stay (3 months) for pilot study on the optimization of the high-rate contact stabilization process at the Blue Plains advanced wastewater treatment plant, Washington D.C., U.S.A. Collaboration with DCWater, Washington D.C., U.S.A.

2013-2014 Long-term follow-up of microbial community at the Nieuwveer wastewater treatment plant, Breda, The Netherlands. Collaboration with Waterschap Brabantse Delta, The Netherlands.

DANKWOORD

Na mijn eerste dag als kersverse doctorandus grapte ik dat al 0.07% van mijn doctoraat achter de rug was. Nu heb ik opeens de 100% bereikt, en het kwam sneller dan ik gedacht had. Na vier jaar klimmen op een berg waarvan de top altijd ver verwijderd leek, blijk ik hem opeens bereikt te hebben. Ik heb tijdens deze tocht hulp gekregen vanuit verscheidene hoeken voor het bereiken van mijn doctoraat, dus wil ik graag een aantal mensen bedanken.

Vooreerst mijn promotoren Siegfried en Nico. Siegfried, toen ik mijn doctoraat startte was je nog volledig postdoc in LabMET. De tijden veranderden. Je werd deeltijds professor in Gent, maakte daarna carrière als voltijds professor in Antwerpen, en bouwde daar een nieuwe onderzoeksgroep uit. LabMET werd CMET. Maar van die veranderingen was niets te merken tijdens onze vergadermomenten (tenzij misschien de locatie, die mee verschoof met je functie, van de coffeeroom en de academische club tot je eigen bureau en uiteindelijk zelfs ‘s ochtends op de trein naar Antwerpen). Steeds bleef je even toegewijd aan mijn onderzoek. Met oog voor detail heb je nagenoeg alles nagelezen wat ik ooit op papier gezet heb, telkens met aandacht voor zowel de kleine schoonheidsfoutjes als de grote lijnen, voor zowel de correctheid als de ‘catchiness’ van het verhaal, en voor zowel de wetenschappelijke volledigheid als de gewenste impact op het publiek. Voor elke thesistudent die ik onder mijn hoede kreeg, probeerde ik hetzelfde te doen, maar op zoveel niveau’s tegelijk naar een tekst kunnen kijken is mij nooit helemaal gelukt. Regelmatig stuurde je mijn teksten terug met de boodschap dat het ‘nog wat scherper’ moest, en steevast moest ik je gelijk geven. Ook van sommige andere van je kwaliteiten kan ik veel bijleren. Als meester-communicator werkte je tijdens sociale evenementen niet alleen aan het uitbreiden van je eigen netwerk, maar dat van je hele team, en dat siert je. Als topdiplomaat slaagde je er tijdens vergaderingen telkens in voor alle partijen een win-win scenario op poten te zetten (of het toch zo te doen lijken), en dat bewonder ik. Het professorschap is je op het lijf geschreven, maar in een parallel universum had je een uitstekend politicus kunnen worden. Dat we nog veel mogen samenwerken!

Nico, jij was de tegenpool in dit duopromotorschap. Bij jou staat de liefde voor de wetenschap op de eerste plaats, alle rest is bijzaak. Altijd was er wel een interessante paper die je had gelezen en eens op mijn project wilde betrekken, altijd was er wel een fundamenteel experimentje dat je mij wilde voorstellen om één van je theorieën te testen. Met een lichte afkeer voor formaliteiten wilde je zo veel mogelijk uit je tijd halen om aan echte wetenschap te doen, en dat is inderdaad waar het om draait. Ik vond het heel fijn om samen onze Breda-paper in elkaar te knutselen (Chapter 2). Door de speciale opbouw van de paper rond vier hypothesen was het erop of eronder of de reviewers dit zouden accepteren, maar het is van de eerste keer gelukt! Ook het vernieuwen van de labopractica van Microbieel-Ecologische Processen was een aangename taak. Elk jaar wilde je weer loskomen van de oude structuren, zodat de studenten nieuwe dingen konden uitproberen. In de “Day To Day” meetings verleende je telkens enthousiast steun aan nieuwe initiatieven van onderuit, of het nu ging om duurzaamheidsinitiatieven of het organiseren van een evenement voor communicatie naar het brede publiek. Je creativiteit is het grootst tijdens momenten van overleg en dialoog, en dat zorgde ervoor dat nagenoeg bijna elke vergadering een brainstormsessie was, ongeacht hoe ver het project al gevorderd was. Ik moest altijd zorgen dat ik genoeg papier mee had om nota’s te kunnen maken

op het moment dat je zou zeggen “maar wacht, als we het nu eens *zo* doen!” Je begeestering werkt aanstekelijk, en uiteindelijk kon je mij zelfs overtuigen om de verschillen tussen Gentenaren en Antwerpenaren uit te leggen tijdens mijn doctoraatsverdediging. Kortom, nieuwe wegen durven inslaan, nieuwe verbindingen leggen, steeds verder denken dan de meest recente literatuur, …, zelfs als rasechte wetenschapper heb je nog nooit gehoord van een ivoren toren. Met jou aan het roer ben ik zeker dat CMET nog lang een bepalende koers zal voeren in de internationale wateren van de éénentwintigste eeuw.

I want to thank my jury members for carefully reviewing my PhD manuscript. Gavin, Haydée, Kristel, Stijn, and Ingmar, due to the way the internal and public defenses were planned, you only had a few weeks to read my manuscript, and I had almost two months to respond to your comments. Still, your valuable suggestions helped me improve the quality a lot; it definitely is a privilege to have your work read by five experts in the field. Paul, thank you for taking up the task of chairman. I was lucky that my jury members were so flexible to have my public defense on a Friday - I know it wasn’t ideal for everyone. I hope we will be able to collaborate again in the future.

Professor Verstraete, u mag dan wel geen promotor geweest zijn van mij, maar ik wil u toch bedanken om de vlam aan te wakkeren die tot mijn doctoraat leidde. Als biologiestudent kwam ik toevallig op de LabMET website terecht en zag ik de overvloed aan milieu-gerelateerde onderwerpen waar u toen onderzoek naar deed. Ik was meteen overtuigd dat ik mijn thesis bij de bio-ingenieurs wilde maken, en u schreef samen met mij een onderzoeksonderwerp uit. Ik had het geluk deel uit te maken van de laatste cohorte thesisstudenten die onder uw inmiddels legendarische begeleiding viel. Ik begrijp nog steeds niet hoe u de tijd vond om met iedereen in het labo, inclusief thesisstudenten, maandelijks persoonlijk samen te zitten. Na mijn thesis ging ik een jaar in het buitenland studeren, maar zelfs daarna bleef ik overtuigd dat ik bij LabMET wilde werken, en vatte ik mijn doctoraat aan. Bedankt voor de blijvende interesse in mijn onderzoek en de vele schouderklopjes die ik intussen nog heb ontvangen.

Bij een doctoraat komen veel technische en administratieve zaken kijken. Dankzij het secretariaat en ATP ging dit echter heel vlot. Christine, hoewel de budgetten krap zijn en de toewijzingen steeds meer gereguleerd worden, heb ik me dankzij jouw vakkundige hulp nooit zorgen moeten maken over financiële kwesties. Regine, bedankt om de talrijke lokalen te boeken, gaatjes te vinden in de agenda’s van de proffen, theetjes te brengen tijdens examentoezichten, en papers op te zoeken waar ik geen toegang toe had. Ik dacht dat je het beu zou worden na de honderdste keer, maar ik heb nooit iets anders gezien dan een glimlach. Greet, je was steeds een zeer aangename aanwezigheid in het labo, en het was een plezier om met jou de grote en kleine verbeteringen aan te brengen in het analytisch labo. Mike, ik heb veel aan je bureau gestaan met praktische problemen, en je vond er telkens een oplossing voor. Het was fijn om de Arduino controller in elkaar te knutselen, ook al duurde het twee maanden voor we het ding aan de praat kregen, en ging het na zeven maanden weer kapot. We hebben in elk geval veel geleerd over wat er allemaal kan misgaan met een Arduino controller. Tim, ik sta versteld van wat je allemaal doet: VFA stalen analyseren voor de helft van het

labo, DNA extracties, PCR's en Illumina tests lopen voor de andere helft van het labo, tegelijk experimenteel advies geven over al die moleculaire mumbo-jumbo, de website bouwen en onderhouden, logo-ontwerp en grafish design, de PR helpen verzorgen, en fancy wetenschappelijke figuren maken voor onze publicaties. Dat kan toch allemaal niet in je jobomschrijving gestaan hebben? Renée, bedankt om vier jaar lang mijn vuile afwas te doen en toch te blijven lachen! Siska, bedankt voor al die keren dat ik te laat was om een bestelling te plaatsen, en je het toch nog behandelde in een spoedprocedure. Robin, door jou leerde ik (on)handig omspringen met werkmateriaal. Bedankt om mijn eerste reactoren in elkaar te knutselen, en om elke klus al na een halve dag geklaard te hebben, ook al dacht ik dat het een week zou duren. Sarah, ik werd er iets te vaak naar mijn zin uitgepikt als 'vrijwilliger' om een nieuw tallying-formulier uit te testen, of de precieze kostprijs van een of andere analyse te berekenen, maar dankzij jou staan we nu een heel stuk verder. Industriële projectjes heb je telkens vlot, efficiënt en professioneel gemodereerd, waarvoor dank! Jana en Tom, tijdens de middagpauzes en op weekend was het altijd extra tof met jullie erbij.

Een doctoraat zou maar een saaie bedoening zijn zonder collega's. Oliver, met jou ben ik samen begonnen en (ongeveer) samen geëindigd. We werden ingedeeld in het verst verwijderde bureau van heel LabMET, de post-rotonde, waar plaats was voor zes personen maar waar we alleen zaten. Door opeenvolgende verhuizingen en verschuivingen heb ik op vijf verschillende plaatsen gezeten, maar op het laatst kwamen we weer naast elkaar terecht in de rotonde. Die eerste en laatste plaats waren het leukst. Ik sta er versteld van dat je erin geslaagd bent om je doctoraat te combineren met een halftijdse job; ik heb dat op het einde één maand moeten doen en dat was meer dan genoeg! Je mag dan wel niet altijd aanwezig geweest zijn, je zorgde altijd voor sfeer, en de Nitrogenius-barbecues bij jou thuis waren elk jaar een voltreffer.

Dries, jij was mijn *partner in crime*, of het nu ging over het in leven roepen van een nieuw labootje, onze eigen 15°C kamer, en het daar gezellig te maken door rekken in elkaar te steken die achteraf te hoog bleken te zijn voor het plafond, het organiseren van duurzaamheidsinitiatieven, het op zoek gaan naar een CMET sustainability award in de krochten van de A-blok en daar flessen whisky te ontdekken, het inrichten van studentenclusters, initiatieven te bedenken voor Transitie UGent, of gewoon over de koffieloze koffiepauzes om te babbelen over reactoren, promotors en mid-PhD dipjes. Ik weet niet wie van ons twee het vaakst de kelder heeft moeten dweilen, maar er zal niet veel verschil op gezeten hebben. Ik zie je nog staan als thesisstudent, toen ik in mijn eerste jaar zat, op een opstapje om je schoenen te beschermen, terwijl ik de kelder dweilde omdat mijn reactor overstroomd was. Maar die schroom viel snel weg; de volgende keer stond je lustig mee te dweilen, en langzaamaan werden we ook *partners in crime* in het opkuisen van overstroomde reactoren, zonder daarbij vragen te stellen over het hoe of waarom. Er kan nu eenmaal veel misgaan als je continu water rondpompt in je reactoren, en het is fijn om een collega te hebben die dat begrijpt. Je was een topcollega, en een nog betere vriend. Sorry dat ik per ongeluk je anammox-reactor heb doen falen.

I also owe much gratitude to my other colleagues. Jo, I think you proofread all of my papers, and I had to admit that, after a while, I adapted my writing style in anticipation of your comments, because I knew you would add a bunch of *Oxford commas* anyway. Thanks a lot for organizing my micro-symposium. Kim, thank you for the nice lunch breaks where you would feed me pieces of cucumber and tomato. I never had so much fun in the lab as when we did DGGE's together. Despite the constant hilarity, the results turned out quite good, surprisingly. Curro, thanks for the many fine beer clusters, and the general support. Sylvia, it was nice applying for FWO funding at the same time, going on an international internship at the same time, and finishing our PhDs at the same time. Cristina, it was fun visiting the different WWTPs in the Netherlands. I hope we can successfully continue our DAF project in the coming year. Thanks to Jan for the friendship, Marta for sharing your experience when I just started, Amanda for throwing great parties, Emma for the delicious desserts like American flag cake and Halloween fingers, and FM for the statistical advice and for putting way too much effort in answering my questions on anything science-related. Thanks to Ralph for showing me how to play the berimbau, Erika and Ramon for keeping up the spirit in the lab and beyond, Melanie for promising me to play the flute during the seminar and then never doing it, Hugo for the breakdance demonstrations, and Cristina for being our Nitrogenius-mom. Jianyun, I'll never forget how you took the time to show me how to pour plates when my tutor didn't have time, even though I was an unknown thesis student to you. Thanks to Stephen for being unorthodox, Mathias and Wout for forcing me to improve my humor, Charlotte for being cheeky, and Joeri for being sharp. Nicole, I loved the walks in the Bourgoyen, where we dreamed about going to New York. Thanks to Delphine for the big smiles, Floor for making me realize we don't get paid enough, Ruben for the entertaining ice hockey stories and for helping me improve my graphics in R (Figure 2.2), and Eva for being my only fellow biologist in the lab when I started, and secretly forming a team against everyone else. Tom, I admire your drive. It's confronting to see someone operating seven reactors at the same time, guiding a few students, and still find time to be part of several commissions for the benefit of the lab and the faculty. Jessica, thanks for proofreading my correspondence to Germany and telling me my letters were "good for a first try", even though they were actually written by Oliver, another native German speaker. Sam, thanks for the help with flow cytometry, and for being a man with strong principles, which I always admire. Thank you, Abbas for showing me around at the University of Antwerp, and for buying me drinks even though you don't drink alcohol yourself. Sam, Maarten and Danny, it was a lot of fun writing popular-scientific articles and blog posts with you. Let's keep doing that! Emilie, it was fun working together on the firewater project. Adrian, you showed me that it's possible to stay forever young. Joachim, I appreciate your kindliness and humor, and Gio, I appreciate your openness and joviality. Baharak, I love your *joie de vivre*, and it's an honor to be your MFV (my favorite vegetarian). However, we both know that the real honor should go to your other MFV, Andreas, whose untimely death shook our lab. Emmy, the vegetarian lunches you organized outside the faculty were always a pleasure. Ramiro and Soupi, you belong to the rare people to ever convince me to eat meat, because of your legendary dinners.

I'd like to especially thank all of my colleagues again for helping to clean up the flooded basement during my first year. I will never forget how the majority of the lab spent their day cleaning up the mess I caused, and, hopefully, I will never again forget to turn off the tap before I go home.

I also had the privilege to be part of a number of 'teams'. Thanks to the Nitrogenius group, the N-tech cluster, and especially the AB-cluster, which has unfortunately gone extinct now, my work has received a lot of valuable input. Dries, Rosemarie, Charlotte, Eline and Floor, we were able to achieve some meaningful changes with the CMET sustainability team. Nico, Christine, Mike, Tim, Jan and Abbas, you were an efficient team to solve the small and large problems of the lab in the D2D. Bart, Tim, Eleni and Chris, in the LabMET-to-CMET transition team, we thought we would get everything organized in a few weeks, but it took more than a year. However, this team was a wonderful example of the democratic and open decision structure of CMET, and that's what makes it a great place to work. Jana, Chris, Joachim and Caroline, I was late to join the faculty board, but you were a super team to work with. Chris, Sam, Simon, Mike, Frederiek-Maarten, Kristof and Oliver, I absolutely enjoyed organizing the practicals for Microbial-Ecological Processes with you. Even when the experiments didn't always turn out quite right, I believe we were able to 'pass the bug' of microbial research on to some of the students. The practicals were responsible for some hilarious moments, such as when a group of students picked up a dubious species of bacteria in their bathroom, or when the student evaluations said that "the assistant's wardrobe only contains two shirts: a blue one and a black one." Maybe buy a white shirt for next time, Jo? Also, many thanks to Greet for preparing all the materials and running the analyses. Marlies, Jan, Jo, Dries and Pieter, as the jury panel for the assignments of Biotechnological Processes, we have scared a lot of students, but for us, it was enjoyable. I'm sure none of them will ever forget that methanogens are Archaea, not Bacteria.

Een doctoraatsstudent zonder thesisstudenten is als een IC zonder autosampler: het gaat wel, maar het is saaier en veel minder efficiënt. Hoewel het een proces van vallen en opstaan was, heb ik er zeer van genoten om studenten te begeleiden. Jensen, als mijn eerste student na amper enkele maanden bezig te zijn, heb ik misschien even veel van jou geleerd als jij van mij. Als een goed geoliede machine liepen we de ene batch test na de andere, en leerden we enorm veel bij - was het niet over hoe het HiCS proces werkt, dan wel over hoe een batch test eigenlijk gelopen moet worden. Het flatteerde mij dat je jaren later, als milieuadviseur, nog steeds mijn advies kwam vragen over waterzuivering en over hoe je problemen met Word oplost. Tim, Stijn en Jona, de eer om drie studenten tegelijk te begeleiden tijdens mijn tweede jaar was bijna teveel van het goede, want was ik klaar met één iemand te begeleiden, dan kwam de andere alweer om feedback vragen. Ondanks mijn tijdsgebrek hebben jullie drie goede thesissen afgeleverd, en de levendigheid waarmee jullie participeerden in de AB-cluster waren een plezier om mee te maken. Tim, een student die bruist van de ideeën is goud waard, en dankzij de discussies met jou zijn mijn experimenten een heel stuk verbeterd in kwaliteit. Ik herinner me dat we microscoopfoto's wilden maken, en de camera van de microscoop kapot was. Je positioneerde toen je eigen spiegelreflexcamera met statief voor het oculair van de microscoop, wat uiteindelijk verscheidene uren werk heeft gekost, maar de beelden waren mooi. Eén van deze foto's heeft het geschopt tot de coverafbeelding van dit doctoraat. Ik ben

enorm dankbaar voor het wekenlange vrijwillige werk dat je kwam doen na je thesis, wachtend tot wanneer je je eigen doctoraat kon beginnen. Je bracht mijn werk onder de aandacht in Washington, waar men het werk verderzette door pilootexperimenten op te starten, en opende zo de deur voor mijn onderzoeksverblijf aldaar. Die drie maanden in Washington, samen met de road trip die we later deden vóór de conferentie in Denver, waren onvergetelijk. Bedankt! Koen en Veerle, tijdens mijn derde jaar vormden jullie, samen met Tim, een hecht team van HiCS-experts. Ik was fier op jullie professionele inbreng in de studentencluster. De dagen dat we met dit team de mysteries van het HiCS proces probeerden te doorgronden moeten de fijnste van mijn LabMET-carrière geweest zijn. Hoewel ik weer heel wat overstroomde reactoren moest helpen opdweilen - het lijkt zowat de enige constante te zijn in mijn doctoraat - miste ik jullie levendigheid toen ik in mijn laatste jaar geen studenten meer had. Ook een dikke merci aan Bavo om de vele liters synthetisch afvalwater te helpen maken, ook al was je niet mijn student.

Ook mensen buiten het labo ben ik veel dank verschuldigd. Prof. Verliefde en prof. Nopens, bedankt voor het gul ter beschikking stellen van jullie labo's en meetapparatuur, en bedankt ook aan Quenten, Tinne, Chaïm en Giacomo om uit te leggen hoe die meetapparatuur dan wel gebruikt moest worden. Dank aan Hans en Leonie voor de vele uren hulp met het verzamelen van informatie over de waterzuivering in Breda.

A big thanks to the people in DC Water for guiding me during my research stay in Washington. Sudhir, Ahmed and Haydée, it was an honor to be part of your research team, and thank you for the valuable input. Haydée, I admired your quick thinking and extreme focus during our meetings. Thanks to your guidance, our understanding of the HiCS process increased tenfold. Arif, you are an incredibly hard worker, and I admire your endurance. Thank you for the fruitful collaboration, and, more importantly, the friendship. Abdul, I enjoyed your sense of humor and our long discussions about gun laws, the effect of sludge seeding on SRT, and the best way to make a peanut butter jelly sandwich. I don't understand how you managed to convince me to participate in an overnight sampling campaign for your experiment the same day that you asked me, but I actually had fun. Shravani, your cheerfulness was contagious. Thanks for the great food, the parties and the drinks. Heather, you were responsible for wonderful moments of liveliness in and outside of the lab. Thank you for tirelessly showing us around in Denver during the conference. Manel, I had a great time staying in your downtown apartment, and some of the moments in the lab were hilarious. Mofei, Tri and Qi, thanks for the great weekend trips, and for letting me try unknown Asian foods. Norman, I enjoyed listening to your endless stream of knowledgeable facts, and thanks for the weekly lunches on Chinese Tuesdays.

En dan zijn we bij mijn vrienden aanbeland. Hoewel veel collega's in de voorbije jaren ook vrienden geworden zijn, heb ik nog heel wat andere mensen te bedanken die voor afleiding, sociaal contact en motivatie hebben gezorgd tijdens mijn doctoraat. Graciela, Jozefien, Kathleen, Karen, Thomas, Tanguy, Isabelle en Elise, met UltraViolet hebben we heel wat mooie momenten beleefd. We hebben de band opgestart op hetzelfde moment dat ik mijn doctoraat startte, en ik vond het heel

mooi om mijn doctoraat ook af te sluiten met een optreden door jullie. Dankzij jullie heb ik, als beginnende cello-student, heel wat bijgeleerd op muzikaal vlak, maar nog veel belangrijker is de fantastische vriendschap die ermee gepaard gaat. Dat er nog vele optredens mogen komen! Niels, dank je wel om het design van mijn coverpagina onder handen te nemen. Kim, Thijs, Sofie, Chris, Benjamin, Jelke en Tess, ik weet niet hoe ik in dit vriendengroepje van ex-LabMET studenten verzeild ben geraakt, maar de spelletjesmiddagen, badmintonsessies, filmavondjes en natuurlijk de reisjes waren onvergetelijk. Ik kan trouwens geen mooiere plek bedenken waar ik dit dankwoord had kunnen schrijven dan op reis met jullie in Sicilië. Eline, Lore en Ellie, de laatste tijd zagen we elkaar veel te weinig, maar de dinertjes, uitstapjes en drinks die we hebben voortgezet uit onze studententijd, zorgden altijd voor dat extra beetje afleiding en motivatie. Tot snel! Ook een dikke merci aan de bende biologen voor de toffe biologenweekendjes. Lyn, we zien elkaar ook iets te weinig, maar als we dan eens afspreken, is het weer alsof we in het middelbaar zitten, avontuurlijk en onschuldig. Mattias en Roxanne, bedankt voor de mooie vriendschap al die jaren, de fijne etentjes en de fantastische weekendjes. Ik kijk weer uit naar onze volgende fossielenzoektocht op de Cap Blanc Nez! Walter, Wouter, Xavier, Frank en Hannes, in ons filosofisch leesgroepje word ik er steeds weer aan herinnerd dat er belangrijkere dingen zijn in het leven dan materialisme en conventies. Max, your utter indifference whether I did a PhD or not, as long as I live my life like an ethical human being, may very well have kept my feet on the ground. Danny en Sarah, ik heb enorm genoten van onze weekendjes in Boston en Washington, en het is fijn om te kunnen zeggen dat ik op Harvard gewerkt heb, al was het maar één dag. Luc en Birgit, het is fijn om jullie steeds beter te leren kennen. Bedankt voor de champagnesponsoring op mijn doctoraatsreceptie!

Some of my friends from Stanford continue to inspire me until today, and I would like to thank them for keeping me ambitious. Fatima, I don't think I've ever met someone so idealistic and crazy about science. You astonished me by dropping out of your PhD at Stanford to start another PhD at MIT. I had a great time in Barcelona, and hope we can meet again soon. POF, I don't think I've ever met someone so talented, being an engineer, polyglot, and organ virtuoso at what, age 21? I had a great time in Paris, let's repeat that someday. Howard and Jane, I am forever inspired by the selfeless effort you put into taking me into your home, and showing me San Francisco and American life in general at, if I'm not mistaken, age 79. Keegan, your friendship meant a lot to me, and completed the 'American experience' I was looking for. Wishing you all the best in your marriage! Raf, Stéphanie and Nicholas, as fellow expats, you cultivated a family-like atmosphere and created the 'Belgian experience' I apparently missed. Valerie, did I win the race to be the first one to publish in ES&T? Anyhow, I leave you the honor of being the first one to become professor :) Alejandro, stay young and full of wonder. Ankit, you showed me that unconditional friendship does exist. Austin, you showed me that life is what you make of it, so you better make it worthwile. Maybe philosophy can be applied after all? As I become doctor of philosophy, I will try to apply what all of you taught me.

De mensen die het belangrijkst zijn voor mij, behoeven misschien wel de minste uitleg. Mama en papa, ik moet jullie bedanken voor bijna alles. Ik kan soms koppig zijn en mijn eigen gedacht volgen. Ik ben dan ook dankbaar dat jullie mij al heel vroeg de ruimte gegeven hebben om zelf keuzes te

maken, en mij daarin toch onvoorwaardelijk te steunen. Elise en Andreas, jullie zijn een fantastische zus en broer. Ik had mij echt geen betere familie kunnen indenken dan jullie! Leo, je past mooi in het rijtje van familieleden met allemaal een totaal verschillend karakter! Dat er nog maar veel toffe etentjes en reisjes mogen komen. Niels, in die laatste helft van mijn doctoraat heb je altijd voor mij klaargestaan. Ik zie je nog synthetisch afvalwater helpen maken, die keren dat je mij in het weekend naar de faculteit bracht om mijn reactoren te onderhouden. Ik hoop dat ik even veel voor jou kan betekenen als jij voor mij. Ik zeg het misschien niet vaak, maar ik zie je graag.